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NITRATE AND CARBOHYDRATE RESERVES IN RELATION TO NITROGEN NUTRITION OF PINEAPPLE

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(WITH FOURTEEN FIGURES)

Introduction

Chemical analyses of leaves and other organs of horticultural crops for nitrogen, phosphorus, and potassium have shown that often—with critical selection of samples to be analyzed—there may be obtained reasonably accurate indices of the concentration of these nutrients in the plant. As applied to agriculture, this has often led to increased efficiency in the use of fertilizers (**3, 4, 11, 27, 32**). If employed as a practical guide to fertilizer requirements, however, such analyses involve the assumption that leaf values for N, P, and K that were correlated with best yields in any year or series of years should be considered as standards in successive years, at least in the same location.

Perhaps when water is not a limiting factor, and if there are locations where temperature and sunshine do not vary greatly from year to year, such an assumption may be adequate for practical purposes. In Hawaii, however, even in the same section of any given plantation, the amount of nitrogenous fertilizer required for maximum yields of pineapples has been found to vary by as much as 75 per cent in successive years. The nitrate reserve which must be present in the leaves of the plants for best yields varies in different years and in the same year in different locations. Indirectly, the reason for this lies in the fact that the plants are grown under conditions of temperature that differ materially, at elevations from only a little above sea level to over 2000 feet. Light intensity varies from less than 2000 to about 8000 foot candles in different seasons and locations. In spite of these differences, and sometimes radical variations in rainfall, it has been possible to obtain quantitative records that, regardless of location and weather, furnish the

information necessary for precise, efficient use of fertilizer. That these plant records are not limited in their use to a single location or season is owing primarily to the fact that carbon dioxide is regarded as a nutrient, and that quantitative records of starch reserves are employed as an index of carbon dioxide availability. All nutrients are essential, of course, but, with the exception of water, carbon dioxide is by far the most abundant nutrient the plant receives.

The uncertainty of the degree of availability of carbon dioxide makes records of carbohydrate reserves in the growing plant particularly pertinent. It is just as essential to have a measure of stored starch as it is to have it of nitrate, phosphorus, and potassium. In fact, starch reserves determine the percentage of nitrate that may be advantageously carried in the pineapple plant. In turn, for greatest efficiency, phosphate levels in the plant should be adjusted on the basis of nitrate concentration, and similarly nitrate is not freely absorbed if potassium is low, etc. Thus there is a chain of interdependent relationships directly or indirectly associated with opportunity for carbon dioxide assimilation that necessarily varies with prevailing weather conditions.

Although for best economic results the importance of avoiding an imbalanced relationship between nitrate, phosphate, potassium, and other ions in the plant cannot be overemphasized, the present report is concerned largely with carbohydrate and nitrate reserves. The principles involved have been discussed in part by KRAUS and KRAYBILL (**10**), and methods of applying these principles to large-scale pineapple production over considerable acreages were developed, first in sand or water cultures and in some cases under controlled environmental conditions, with tomato, fruit trees, and other horticultural crops (**16, 17, 18, 19**). These principles, applied to pineapple, have shown that the concentration of nitrate which the plant is permitted to accumulate should be adjusted in relation to its starch reserves. A deficiency of either plant constituent is to be avoided. Standards for judging the amounts present in relation to any given functional level of the plant are to be described.

Together with climatological data, various records of plants are also taken, such as starch and nitrate determinations. The data indicate why a particular field or area may include plants deficient in starch, nitrate, or other nutrients. If plants are deficient in any essential constituent, it becomes important to know why. It is not enough simply to know that the condition exists. For example, if nitrate reserves are low because of suberized root tips and root hairs and consequent limited absorptive capacity, the practical indications are in contrast to that of a field with similar nitrate reserves in the plants but with root systems capable of more active absorption. Thus the purpose of quantitative records of the growing plants is primarily to measure limiting factors, not alone those that can be controlled or in-

fluenced, but also those that cannot be controlled. Such records permit purposeful, well-timed applications of fertilizer. They also help to prevent futile attempts to attain impossible objectives.

Observations

Quantitative field records

In this initial paper—dealing with the field aspects of pineapple nutrition—that may be referred to in later reports, it is proposed to give a rather inclusive description of methods of obtaining records of plants in the field. A mature pineapple plant of the Smooth Cayenne variety is shown in some detail in figure 1. It is propagated vegetatively and is almost the only variety of commercial importance in Hawaii. Figures 2–5 show plants of various ages as they appear in the field: planted through mulch paper, in 2-row beds 100 yards in length, the beds terminating at a field road at each end.

Representative samples of the distal half of the stem of the slips when employed for planting are analyzed for percentage dry matter (a good index of carbohydrate reserves and capacity for root production), for phosphorus, potassium, and nitrate. These constituents may be stored in quantity in the slips, although the amount and proportions may vary greatly with different lots of planting material. As soon after planting as roots have become established and some new top growth made, quantitative records are taken at about monthly intervals until blossom buds appear about a year later. The time from planting to the appearance of blossom buds may vary greatly, depending upon many factors.

Plant weight.—In a productive commercial pineapple field, plants vary relatively little as compared with many horticultural crops. Nevertheless, in any part of a field under consideration, three plants are selected. These are judged, for the particular date concerned, to be large, medium, or small in comparison with the remainder of the plants in the area. These plants are pulled up and weighed.

Number of leaves.—The number of leaves over 12 inches long on each of these three plants is recorded.

Length × width of leaf.—A leaf is selected for measurement on each of the three plants. Figure 6 illustrates on the left an immature leaf; on the right, one that is approaching senescence. Between these is a leaf, representative of those selected for measurement, that is almost fully expanded, except for the lateral points at the base. The width is determined at a point one-third the distance up from the base of this leaf. Because a leaf persists for many months, leaf dimensions tell little of the quality of the plant, but they do serve as an index of the growing conditions at the time the leaf concerned was expanding.

Selection of plants.—In several key locations in a field, the following records are taken from a pair of representative plant beds. Proceeding along the cultivated

FIG. 1.—Plant at about 21 months; leaves cut off to show axillary shoots which produce second crop, the first ratoon. (Axillary shoots may be employed for planting, together with so-called slips.) Small slips just visible along peduncle. Small knoblike rudimentary fruit is discarded from slip before planting.

space, five paces in from the road, a plant from the nearest row in the left-hand bed is recorded as being large, medium, or small, as the case may be. Ten paces farther along, a plant from the nearest row in the right-hand bed is selected, and so on, until the other end of the bed has been reached and ten plants have been

FIG. 2.—Pineapple plants at about 6 months, showing style of planting, through edge of black mulch paper, in 2-row beds with cultivator space between.

recorded, five from either bed. By analysis of variance there is thus obtained a reliable index of plant weight, number of leaves, and length × width of the leaf described (fig. 6), for the beds concerned.

ROOT ANCHORAGE.—When the three plants are being pulled up, a spring-balance is employed to record the number of pounds required to uproot them. On the basis of these tests and experience over a considerable period of time, a moderate tug at each of the ten plants enables an experienced worker to record for each the proximate root anchorage in pounds. This gives an index of the extent of the root

system. Anchorage of 100 pounds is considered 100 per cent, although it may reach as much as 150 pounds.

WHITE ROOT TIPS.—These are indicative of the ability of the plant to absorb water and other nutrients. In several locations, small trenches are dug inside and outside the beds so that the character of the root system may be determined.

FIG. 3.—Pineapple plants at about 9 months. Along the troughlike upper surface of leaves, water from light showers or dew may flow toward center of plant, down through planting hole in mulch paper, to soil below. Fertilizer placed in lower axils of leaves is thus carried down and made available.

Samples for soil moisture are also taken at this time, usually under the edge of the mulch paper midway between the plants in the row at a depth of 6–8 inches. If all main roots and lateral roots observed have white root tips $\frac{1}{2}$ and $\frac{1}{4}$ inch or more in length, respectively, white root tips are recorded as 100 per cent. Anything less than this is indicated correspondingly on a percentage basis.

SEMI-MATURE ROOT TIPS.—Such root tips, recorded on a percentage basis, are slightly brownish, suberized, and relatively woody up to and often including the apex. They are incapable of adequate absorption of water, at least when evapora-

tion rates are high. Often within a day or two after soil conditions have become favorable, cell division may commence and new white root tips emerge apically. (Factors which singly or in combination may adversely influence the maintenance of active white root tips may be only mentioned here. They include lack of water, as when soil moisture is at or below the wilting percentage, probably high soil temperature [16, 30], and high soil solute concentration as shown by HAYWARD [6, 7] and others [19].)

FIG. 4.—Pineapple plant in blossom at about 15 months after planting

SENESCENT ROOT TIPS.—As implied, such root tips are dead or nearly so. If 100 per cent are in this condition, it means that it has been some time, probably weeks, since the plant has absorbed water and nutrients freely in adequate quantity—regardless of soil moisture content. It also means that, if the distal tissues of the roots are senescent, when soil conditions become favorable new white root tips will emerge relatively slowly, laterally well back on the main roots or as new adventitious roots from the base of the stem.

ROOT FACTOR.—To co-ordinate root condition in a single quantitative expression, the root factor is considered to be root anchorage in pounds × percentage of white root tips. These records of root condition, while only proximately quantita-

tive, play a part in agricultural practice and in the interpretation of responses of pineapple plants in fertilizer experiments that is at least equal in importance to the more precise chemical analyses and other determinations to be described.

LEAF COLOR AND CARBOHYDRATES.—Often in a pineapple plant in which all the potential starch-storing cells are filled to capacity, macroanalyses—expressed as percentage either of fresh or of dry material—indicate relatively little starch.

FIG. 5.—Field nearly ready to harvest at about 21 months after planting

Hundreds of such macroanalyses of stems and leaves have been made, yet there has been no consistent correlation with starch deposits in the leaves that were easily seen by microscopic examination. The tests completely failed to indicate to what percentage of capacity the plant was supplied with starch. The reason is obvious. The pineapple plant frequently contains a very high proportion of inert mechanical fiber in both stem and leaves, and analysis of expressed juice, although of interest, does not include starch, the major carbohydrate reserve. Another plant type, relatively soft and succulent and with limited development of fibers, was—by microscopic observation—very low in starch. Yet as percentage of fresh or dry weight relatively more starch was indicated in such a plant than in the case of one

filled to capacity with this material. Of course, the absolute amount of starch could be easily computed from the conventional macroanalyses; but in nutritional studies, concentration is the index of degree of deficiency of carbohydrates, or any other material. Absolute amounts are correlated with size and not with quality of plant.

On the basis of microscopic tests, it soon became apparent that there was a consistent correlation between degree of greenness and starch reserves in the potential starch-storing cells of leaves. It should be recognized, however, that in a woody, highly differentiated leaf of relatively xeromorphic structure, the number of cells capable of storing starch are comparatively few and their protoplasmic volume usually relatively small. Nevertheless, if all these cells are approximately filled to capacity with starch, as in a yellow-green leaf, the carbohydrate rating is considered 100 per cent for that leaf. As already pointed out, this is in contrast to any figure that could be obtained by macroanalysis, where—on a percentage of fresh or dry-weight basis or as absolute amount in grams—the starch content would be recorded as very low for that leaf. It is recognized that this method of estimating carbohydrate reserves is a departure from the more conventional methods of expression employed by plant physiologists, but in principle it corresponds to expression on the basis of "protoplasmic mass" long used by animal chemists. In any event, this method—together with analyses for nitrate—is useful in practice and gives a high degree of precision.

FIG. 6.—Left: immature leaf with relatively much white semi-meristematic tissue and comparatively narrow sides tapering inward slightly at base. Right: mature leaf with little white semi-meristematic basal tissue; base broad in relation to remainder of leaf, with pronounced lateral points forming acute angles. Center: leaf nearly fully expanded except at base; middle third of white basal tissue of leaves of this type employed for chemical analysis.

COLOR.—The color standards shown in figure 7 are recognizably distinct, but of course they intergrade insensibly. They represent an arbitrary median region of a color range for only the upper surface of leaves. Of this area, certain portions are excluded in making estimates of the leaf color of a plant for starch reserves. The areas omitted include the immature leaves, less than 9 inches long, in the center of the plant, the immature, relatively unexposed proximal third of longer leaves, and the distal third or tip of longer leaves. This terminal tissue is always relatively senescent after the very early stages of growth, and in any event is small in area because the leaf tapers to a point. Thus it is the color-starch relationship of only the potential starch-storing cells of approximately the middle third of the leaf that is referred to here.

NO. 0 COLOR: YELLOW.—This region in the color scale is represented by senescent leaves or senescent areas of leaves. They are yellow or yellowish brown and practically free of any pronounced shade of green. The starch content of such leaves is variable, and it is doubtful whether these leaves contribute materially to carbohydrate metabolism.

NO. 1 COLOR: YELLOW-GREEN.—The median region of this color range is much less green than no. 2. It is decidedly yellowish and characteristic of leaves much exposed to sunlight. Leaves in this color range contain starch to within 75–100 per cent of capacity. Leaves may be injured by excessive sunlight or very high temperature, or both, and exhibit shades of greenish yellow similar to no. 1 color, but can be easily distinguished by characteristic uneven coloring and internally by disorganization of chloroplasts (**8**).

NO. 2 COLOR: OLIVE-GREEN.—The median region of this color range is olive-green. The upper whorls of leaves often exhibit this color as the plant matures. This is particularly noticeable in a sunny season or location, unless the plants are very heavily fertilized with nitrogen, or closely planted, or both. Starch reserves are within 50–75 per cent of capacity in leaves of this class.

NO. 3 COLOR: BLACKISH-GREEN.—The median region of this color range is very dark green. In much shaded, lower leaves it reaches an almost black-green. It is typical of plants under growing conditions when abundant nitrogen has been supplied or when subjected to relatively cloudy weather, or both. The starch content of leaves of this color is 25–50 per cent of capacity.

RECORDS OF LEAF COLOR.—Recording the color of the leaves is considerably simplified by the fact that there are usually rather definite zones of greenness. Invariably the lower leaves are darker green, midway along the stem the leaves are intermediate in greenness, while the upper whorls are comparatively less green. For each of the ten plants selected along the length of the two beds there is recorded for the plant as a whole the percentages of the leaf area that on the upper surface may exhibit one or more of the classes of color shown in figure 7, very young

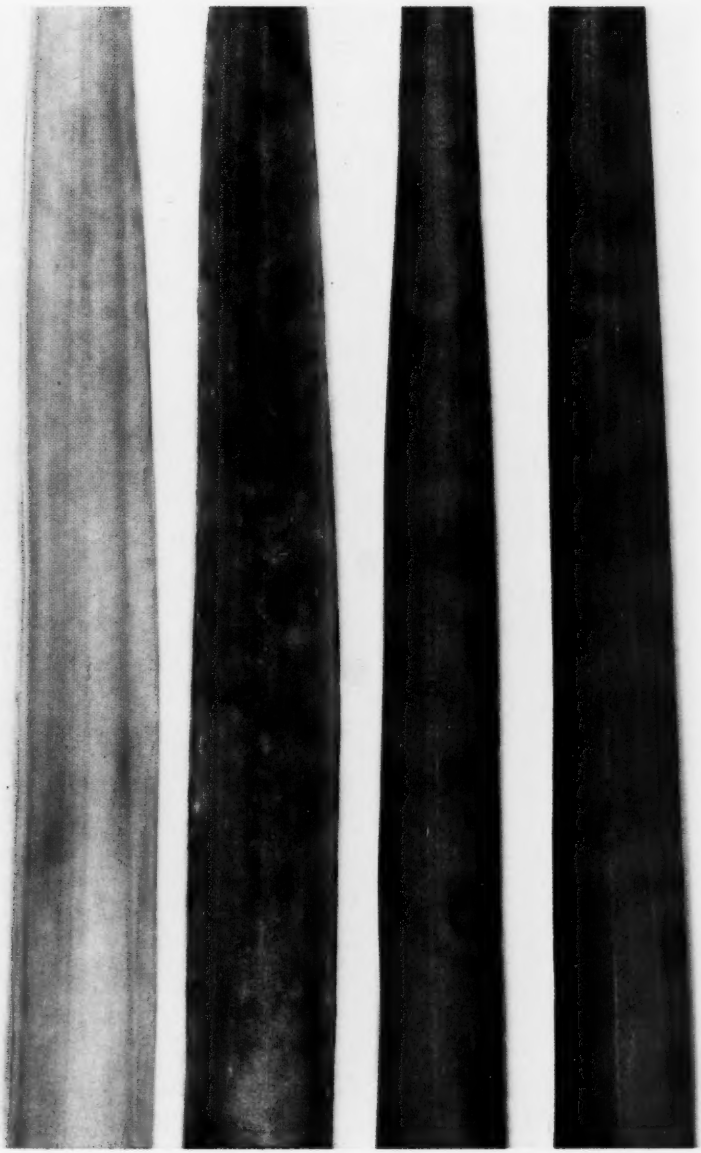

FIG. 7.—Median region of four ranges of color of pineapple leaves. From left to right: no. 0, yellow; no. 1, yellow-green; no. 2, olive-green; and no. 3, "black"-green. With reference to maximum possible fruit production, no matter how low or how high nitrate reserves may be, carbohydrate—rather than nitrate—deficiency is indicated and limits yield if in warm sunny location there is less than 15 per cent and in very cool or cloudy location there is less than 30 per cent no. 1 yellow-green leaf color.

leaves and proximal and distal portions of older leaves being excepted, as already noted. The sum of the percentage of each color class equals 100 for each plant. Results are reported as averages of ten plants. Independent records by men trained in this work seldom vary by more than about 3 per cent in the color average of the same lot of ten plants. Very small differences in percentage of no. 1 color, yellow-green, are correlated with striking differences in fruit production, as will be later shown.

LEAF-COLOR INDEX.—Together with other determinations as a guide to nitrogen requirements, the percentage of no. 1 color stands out as highly significant in many experiments, probably because the upper leaves—relatively high in percentage of such color—are more active than other plant parts in nitrate reduction (**24, 25**). Thus, for certain purposes, the respective color values are kept segregated. On the other hand, a weighted leaf-color index of degree of greenness gives an expression of plant condition that is intimately associated with quality of fruit. The weighted leaf-color index is determined by multiplying the percentage of no. 1 color by one, the percentage of no. 2 color by two, and the percentage of no. 3 color by three. The results are added and the sum divided by three. The resultant figure is the leaf-color index.

RED PIGMENT.—The percentage of bluish-red anthocyanin pigment sometimes displayed on the upper surface of pineapple leaves may be recorded, but the degree of pigmentation commonly fluctuates too rapidly to be a useful index for most purposes. For instance, a change in air temperature, if it persists for a day or two, usually is associated with rather striking changes in anthocyanin content; low temperatures are followed by an increase if other factors remain essentially the same. Likewise, plants subjected to serious deficiency are commonly highly pigmented. Both these environmental influences, together with inadequate nitrate reserve, are often correlated with a comparatively high sugar content in the leaves. Results in harmony with these have already been reported by MAGNESS (**12**) and others for apple trees. Under all observed conditions, however, the ratio of starch to sugars is proportionately high as determined macrochemically when starch and total sugars are expressed as dextrose.

LEAF NITRATE-NITROGEN.—The leaf shown at the center of figure 6 illustrates the precise stage of growth of leaves employed for nitrate analysis. They are almost fully expanded, except for the lateral points at the base which, in a fully mature leaf approaching senescence, form a much more acute angle, and in an immature leaf form approximately a right angle. It is thus simple always to select exactly comparable leaves for analysis, regardless of environmental influences or whether the plant has been growing for 3 months or a year. After blossom buds appear, all leaves on the original stem become mature and no further analyses are made for nitrate. Experimental trials have shown that nitrogen fertilization of

pineapple, after blossom buds develop, is without material influence upon the "mother plant" or fruit. These results are in harmony with the responses of some other monocotyledonous plants, such as oats, as has been shown by WILLIAMS (**33**).

For each of the ten plants used as the basis of records, the leaf as just described is pulled out for nitrate analysis. It breaks off easily and cleanly near the point of attachment to the stem. In this region the white basal tissue is semi-meristematic. It is comparatively homogeneous and contains practically no lignified elements. In several lots of leaves the moisture content in the middle third of the white basal tissue employed for nitrate analysis varied from 89 to 92 per cent. This was true even though the relatively mature portion of the leaf had in some lots a severe water deficit in the specialized water-storage tissue, while others were filled to capacity with stored water, and still others were only partially filled.

Leaves may be harvested for analysis at any time between 8:00 A.M. and 4:00 P.M. At least, tests of comparable plants of several lots indicated no material variation in nitrate content in the leaf owing to the hour of sampling. The middle third of the white basal tissue of the ten leaves from as many plants is minced, mixed, and an aliquot homogenized in water in a small, electrically driven mixer. The amount of nitrate in the clear filtrate, entirely free of any noticeable color, is determined by the phenoldisulphonic acid method (**1**) and is expressed as percentage of nitrate nitrogen. Re-sampling of any plot or bed seldom shows, on the same or following day, a departure from the original results by more than plus or minus 0.003 per cent of nitrate nitrogen.

ROLE OF NITRATE IN THE PLANT.—Leaf-nitrate values, as obtained month after month on tissues strictly comparable physiologically, are directly correlated with the nitrate content of the massive stem of the pineapple plant which may have up to half or more of its total nitrogen content in the form of nitrate. The green portion of the leaf contains little or no nitrate, except under conditions preventing its reduction (**18**). Nitrate does not directly affect the growth responses of plants materially. There must first occur nitrate reduction, oxidation of sugars, and organic nitrogen synthesis; but a sufficiently active rate of synthesis of amino acids and proteins in many plants does not occur unless the tissues concerned contain a liberal reserve of nitrate (**18**). Pineapple is apparently no exception. It is also like most other plants in that it does not store ammonium (**24, 25**), even when ammonium sulphate is practically the only nitrogenous fertilizer employed. Also, under field conditions ammonium is rapidly oxidized to nitrate in the soil. While the pineapple plant can freely absorb and elaborate ammonium (**24, 25**) under field conditions, it seldom—if ever—has an opportunity to do so for a significant length of time.

While any cell constituent may well be considered as exerting some influence on the development of the plant, nitrate is obviously not an essential form of nitrogen,

for—by employing an ammonium salt as the sole external source of nitrogen (**18**) —many species of nitrate-storing plants, including pineapple (**24, 25**), have been grown to maturity and have produced abundant fruit when grown in water cultures with the nutrient solution and the plant itself almost or entirely free of nitrate.

LEAF POTASH AND LEAF PHOSPHORUS.—There are important interdependent relationships between nitrate, phosphorus, and potash in the nutrition of the pineapple plant that will not be discussed in detail in this paper. For determination of total potash and total phosphorus, however, aliquots of the same white basal leaf tissue employed for nitrate analyses are ashed and official methods followed (**1**).

GROWTH INDEX.—This is the weekly rate of increase in length of young leaves, initially 20 cm. long, selected at monthly intervals. The same lots of plants are always employed, care being taken to select representative locations in the field.

IRON DEFICIENCY.—Plants are recorded as being deficient in iron on the basis of the percentage of the leaf area, if any, which exhibits chlorotic mottled areas characteristic of such deficiency. In productive commercial pineapple culture, however, iron deficiency seldom is allowed to persist long enough to be of practical significance, the condition being remedied by spraying with iron sulphate.

ZINC DEFICIENCY.—On the basis of the percentage of leaf area exhibiting characteristic mottled spots or blisters, plants are recorded as being deficient in zinc. Such deficiency is easily prevented by infrequent spraying with zinc sulphate, however, and at present is seldom found in significant degree in commercial fields.

LEAF-WATER DEFICIENCY.—The pineapple plant may not only store very high reserves of starch and nitrate, but in addition the leaves possess specialized water-storage tissue. Macroscopically this is apparent as a clear translucent layer beneath the upper epidermis. Its development proximately keeps pace with differentiation of other leaf tissues. The white semi-meristematic basal tissue of leaves employed for nitrate analysis, being largely undifferentiated, does not include specialized water-storage cells. When the ten leaves from as many plants are collected for nitrate analysis, they are arranged in a bundle with their basal ends placed one on top of another. The bundle of leaves is then cut crosswise, one-third of the distance up from the base, where water-storage cells are fully differentiated. The ratio of the thickness of water-storage tissue to that of chlorophyll-containing tissue, when viewed in transverse section, is then recorded. If the thickness of water-storage tissue is less than that indicated by the following ratios for the appropriate age, it is so reported on a percentage basis.

STAGE OF GROWTH	WATER-STORAGE TISSUE	NONWATER-STORAGE TISSUE
First 10 months	1	2
Eleven months to dried flowers	2	3
After dried flowers	1	1

SUNSHINE RECORDS.—For this purpose, two soil or distance thermographs are employed, the sensitive element of which is a cylindrical copper bulb 12 inches long and about 1 inch in diameter. The bulb of one instrument is placed in a conventional louvered weather shelter; the other bulb is painted black and exposed to full sunlight in a horizontal position, with the length of the bulb in a north-south direction. The difference in temperature between the bulb in the weather shelter and that exposed to sunlight is recorded on the charts of the two instruments. This difference is converted to terms of foot candles on the basis of comparative tests with several instruments for recording sunlight. Results are expressed as the average weekly light rate in foot candles from sunrise to sunset, employing formulas developed by FARDEN (**5**). These instruments are sufficiently sensitive for the purpose employed. In different locations and seasons, the average weekly light rate varies from less than 2000 to about 8000 foot candles.

AVERAGE 2-HOURLY AIR TEMPERATURE.—In its relation to the nutrition of the pineapple plant, the most consistently useful, single expression of air temperature has been the average 2-hourly day-and-night records from thermographs. On the other hand, it is often important to segregate the average day and night temperatures, for two fields may have the same average 24-hour temperature, but if the nights are relatively warm, the plants in that field will be much lower in carbohydrate reserves than in the other if conditions in both locations—including sunlight—are otherwise similar. Past weather records for a location are of considerable local value in connection with the nitrogen nutrition of pineapple, for they have a bearing upon what may reasonably be expected from a fertilizer application to plants with known root systems and content of starch and nitrate. Plants alike in size and quality, and with adequate soil moisture, will not give the same responses in fruit production to the same fertilizer treatment if there follow different conditions of air temperature and sunlight. These and related factors will be discussed later.

FRUIT EXPOSURE.—About a week before fruit is to be harvested, the various plant records are taken as described, except analyses of leaves for nitrate, potassium, and phosphorus. At that time there is also recorded the percentage of total possible exposure of the fruit to direct sunlight. This might seem a superfluous determination, but fruit temperature in large measure determines acidity and texture of the flesh of the fruit. In turn, fruit temperature varies with that of the air, but particularly with the amount of sunshine and the degree of exposure to it. For instance, if the average fruit temperature during the week prior to picking is high because of a high light rate or a high percentage of exposure to sunlight, or both, the fruit will be very low in acidity. Conversely, low fruit temperature makes for high acidity. The explanation may lie in the well-recognized effects of temperature on rate of respiration and concomitant decarboxylation (**26**).

That air temperature alone is no index of fruit temperature is apparent from the fact that on sunny days a fruit with about 100 per cent of possible exposure to sunlight may attain a flesh temperature of 130° F., with a maximum air temperature of only 73° F. Formulas, not given here, have been worked out for computing fruit temperature on the basis of light rate, air temperature, and percentage of exposure to sunlight. Excepting the crown leaves of the fruit itself, exposure is considered 100 per cent when no leaves at any time of the day shade the sides of the fruit. Exposure is recorded as zero if the sides of the fruit are not exposed to direct sunlight at any time of the day. By employing suitable photographs as standards of comparison for different degrees of exposure between these two extremes, reliable records are obtained.

The temperature of the flesh of the fruit has the influences noted; but in relation to sugar content, the average air temperature—taken at 2-hour intervals from thermograph charts during the week prior to harvest—is a dominant factor. The plant does not store starch or other carbohydrate reserves in quantity in unripe fruit, but shortly before ripening there is a rapid influx of sugar from the vegetative organs to the fruit, the rate apparently being determined in large part by air temperature. Under adverse conditions, when the average air temperature for 2-hour intervals during the week prior to picking is low (68° F.), plants high in starch reserves (in that they produce fruits relatively high in sugars) have a decided advantage over plants comparatively low in carbohydrates. This advantage is negligible, however, under favorable intermediate conditions of 69°–75° F. average air temperature during the week prior to picking.

Discussion of results

The pineapple plants of these experiments were all abundantly fruitful, in the sense that they would be placed in KRAUS and KRAYBILL's class III (10). In no case were nitrate or carbohydrates so deficient that lack of either was associated with nonfruitfulness. Nor were any of the plants more extreme in type of growth and composition than have often been found in the past in fields supplied with a predetermined schedule[1] of nitrogen applications. Thus this report deals with a relatively narrow range of fruitful plant types, with differences in degree of nitrate —or carbohydrate—deficiency that are comparatively small as compared with the extremes encompassed by KRAUS and KRAYBILL's four classes. At the time of differentiation, however, even a seemingly slight deficiency of nitrate as related to carbohydrates was correlated with marked decreases in yields of fruit if not rather

[1] A schedule usually decided upon before a field is planted. It gives the dates when nitrogen is to be applied and the amounts. It is largely derived by averaging the results of several years of conventional fertilizer tests employing six replications of each treatment, analyzed each year for statistical significance, with results varying in different years with the weather, to the extent that there may be a 75 per cent difference in total amount of nitrogen required in successive years.

promptly adjusted. These deficiencies, quantitatively measured in the growing plant, have been found remarkably accurate indices of the amounts of nitrogenous fertilizer that may be required at any time, in relation to carbohydrates, for greatest fruit production.

To eliminate as many limiting factors as possible, other than the known levels of nitrate—or carbohydrate—deficiency being tested, in their relationship to production of fruit, all the experiments were conducted in fields where it has been demonstrated that neither potassium nor phosphorus was lacking. When limiting factors appeared, such as deficiency of iron or zinc, they were promptly corrected by applications of sprays containing these elements. Experimental results from areas lowest in sunshine are not included in this report, for such fields are typically in the rainy mountain sections where the soil, owing at least in part to leaching, is usually deficient in potassium and phosphorus.

Unless otherwise indicated, it should be understood that during the experimental periods under consideration, the percentage of white root tips was sufficient for adequate absorption of water and other nutrients, that root anchorage was 100 pounds or more, and that the soil moisture was above the wilting percentage. After the plants were a few months old, ammonium sulphate, when employed, was applied as usual in the lower leaf axils, and therefore did not become available until there had been sufficient rain to dissolve and carry it to the roots. Accordingly, there is indicated in this report not only the actual date of application of fertilizer but the proximate date when it was made available by rain. Weather records, for the most part, are reported only when they appear to be pertinent. The detailed reports of climate, while of local value, are secondary to the theme of this paper—nitrate and carbohydrate reserves as correlated with yield of fruit. The pineapple plant itself is, in part, an expression of these relationships and a reflection of favorable and unfavorable environmental influences more exact than can be derived from records of environment alone.

The experimental results that follow indicate considerable differences in yields of fruit from plants essentially similar in content of nitrate and starch. Differences in yields were often correlated with differences in size of the plants at the time flower-bud primordia developed. Plant size is influenced by many factors, such as number of plants per acre; number of months of vegetative growth, which varies materially with the season of planting; length of time from planting to emergence of roots, which varies with the amount of rain as well as with the carbohydrate reserves in the planting material, and so on. On the other hand, the temperature during the period from appearance of flower buds until the fruit is harvested is of extreme importance in relation to size of fruit. For example, a plant weighing 16 pounds, deficient in neither nitrate nor carbohydrates, may produce a fruit weighing less than 5 pounds if the weather is cool during the 5–6 months from the first

appearance of blossom buds until the fruit is ripe. In contrast, a plant of equally desirable chemical composition, weighing less than half as much, may produce a fruit weighing more than 6 pounds if the temperature conditions are favorable during this same period. Exact quantitative data of this type are available but are not conveniently included in this report.

Figure 8 shows, for various stages of growth, the proximate maximum percentage of nitrate-nitrogen found in the white basal tissue of the leaf employed for analysis. Occasionally nitrate may attain levels slightly higher than those indi-

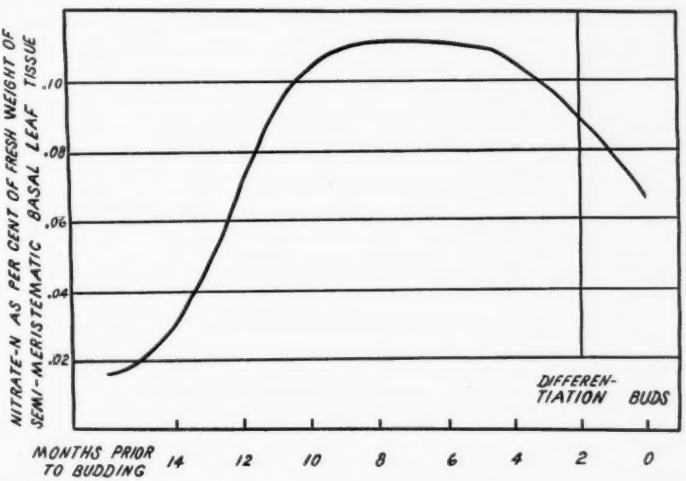

FIG. 8.—Proximate maximum percentages of leaf nitrate-N attainable at different stages of growth. With reference to greatest possible yields, maximum nitrate is desirable only if plants contain adequate carbohydrate reserves, as indicated in warm sunny location by not less than about 15 per cent and in very cool or cloudy location by not less than about 30 per cent no. 1 yellow-green leaf color (cf. fig. 7).

cated, and frequently—if reduction is limited by any factor—will become abnormally high. But it has yet to be demonstrated that increases in yield of fruit have been brought about by raising the percentages of nitrate, for the different periods of plant development, higher than those shown in figure 8. On the contrary, there is evidence to indicate that opportunity for carbohydrate synthesis and accumulation must be exceptional before it becomes desirable to attempt continually to maintain leaf nitrate at the essentially maximum levels indicated in figure 8. Rather it is proposed to show that when nitrogen is added to plants deficient in starch, as indicated by a percentage of no. 1 leaf color (fig. 7) lower than about 15 per cent, yields will not be significantly increased even if leaf-nitrate values are very low for the stage of growth concerned as compared with those shown in figure

8, and even if the nitrate content of the plant is increased. Exceptions to this general rule apparently occur when temperature and sunlight following nitrogen fertilization are unusually favorable for carbohydrate accumulation. In contrast, in locations or seasons of the years, when it is known from past records that weather conditions following a contemplated application of nitrogen to a field are not likely to favor carbohydrate storage (as in cloudy mountain areas not considered in this report), a materially greater percentage of no. 1 color—about 30 per cent—is apparently required before material increases in fruit production occur owing to added nitrogen or absorbed nitrate.

As already pointed out, after blossom buds are well advanced (fig. 4), fertilization with nitrogen has no measurable influence on yield, even in plants extremely deficient in nitrate and containing abundant carbohydrate reserves. At approximately the time of floral differentiation, however, about 2 months before blossom buds become apparent or even a few weeks earlier, plants low in nitrate in relation to starch reserve often respond to added nitrogen with marked increase in production of fruit, if there are no other limiting factors. That the pineapple plant is particularly responsive at this stage of growth may be owing, at least in part, to the fact that it has approximately reached its maximum size, as vegetative expansion of the original plant essentially ceases when the determinate flower bud appears. Thus the total protoplasmic volume available for metabolizing nitrate is probably greater than at any earlier period.

NITRATE DEFICIENCY AT TIME OF FLOWER-BUD DIFFERENTIATION.—It is convenient to consider first the effects on relative yield of fruit of different degrees of nitrate deficiency, maintained for a short period of time, definitely delimited initially by given dates of plant analysis at a little before floral differentiation and terminated finally by emergence of buds (fig. 4), when measurable influences of nitrate on production of fruit cease. It is emphasized that nitrate is not considered deficient, no matter how much lower it may be than the percentage values given in figure 8, for lack of it does not usually limit yields when coupled with deficiency of carbohydrates, as indicated by less than about 15 per cent no. 1 yellow-green leaf color (fig. 7).

From July to the end of August (table 1) the plants decreased in nitrate from 0.090 to 0.063 per cent. In the relatively warm location where they were growing there is a light rate during the summer and fall of typically about 5000 foot candles. Higher nitrate reserves were evidently desirable, for there was no evidence of carbohydrate deficiency, no. 1 leaf color being 21 per cent in August. These nitrate-deficient plants were then fertilized with nitrogen, at that time only in the case of the B and D plots. About a month later definitely higher nitrate reserves were found than in the plants of the A and C series. The D group, with no more nitrogen added (after 120 pounds was applied in September), were thereafter con-

sistently higher in nitrate than the A group, which was unfertilized during the experimental period. Although the D plots were relatively low in carbohydrates, as compared with the A, they had at no time less than 16 per cent no. 1 leaf color and at the time of differentiation displayed 24 per cent. Correlated with these conditions, the D group outyielded the A by more than 3 tons of fruit per acre. That the C plots, not supplied additional nitrogen until late October, yielded slightly less fruit than the D was owing in large part to the fact that the increase in nitrate reserves was not attained until a little later than in the plants of the D plots. As shown in table 1, the plants of the B group were provided (in two applications)

TABLE 1

EXPERIMENT 29 F 3: FIELD 5518, PLANTED NOVEMBER, 1939. YIELDS, POUNDS PER ACRE OF APPLIED NITROGEN, PERCENTAGE OF LEAF NITRATE-N (NO_3), AND PERCENTAGE OF LEAF AREA EXHIBITING YELLOW-GREEN COLOR (NO. 1), WITH REMAINDER OF LEAF AREA NOS. 2 AND 3 COLORS

LOT	PRIOR NITROGEN (LB.)	7/26/40		8/30/40		9/16/40	10/25/40		10/25/40	12/14/40		12/19/40	12/30/40		TOTAL NITROGEN (LB.)	FRUIT (TONS PER ACRE)		
		NO_3	No. 1	NO_3	No. 1	(8/16) NITROGEN (LB.)*	NO_3	No. 1	(10/8) NITROGEN (LB.)*	DIFFERENTIATION†		(11/13) NITROGEN (LB.)*	NO_3	No. 1		TOTAL	PLUS OR MINUS B	REQUIRED FOR ODDS 19:1
										NO_3	No. 1							
A	200	.030	23	.063	21	None	.034	33	None	.051	47	None	.037	51	200	28.88	−3.74	0.71
B	200	.030	23	.063	21	80	.052	21	None	.076	17	80	.073	24	360	32.62	.....	0.71
C	200	.090	23	.063	21	None	.033	28	80	.070	28	None	.059	27	280	31.42	−1.20	0.71
D	200	.090	23	.063	21	120	.057	16	None	.063	24	None	.053	27	320	31.91	−0.71	0.71

* Ammonium sulphate applied in lower leaf axils on dates given in parentheses, but because of lack of sufficient rain to dissolve the fertilizer and carry it to the roots, it was not available until the proximate later date shown.

† Budding occurred about the middle of February.

with a total of 40 pounds more nitrogen than the D group, were consistently higher in nitrate reserves, were at no time deficient in carbohydrates (as 17 per cent was the lowest value for no. 1 leaf color), and—just within the limits of odds of 19:1—they significantly outyielded the D series by 0.71 tons per acre. That the gain in yield was only slightly above that of the D plants was probably owing to the fact that at time of differentiation, when the second application of nitrogen became available on December 14, the plants were nearly filled to capacity with nitrate (fig. 8) and displayed 17 per cent no. 1 color, which is only a little above the arbitrary line of carbohydrate deficiency—considered to be about 15 per cent. Light rate and temperature were such that this did not result in carbohydrate deficiency, however, for even though the plants were essentially filled to capacity with nitrate, they exhibited 24 per cent no. 1 leaf color at the end of December.

The results summarized in tables 1 and 2 for the two experiments in field 5518 show that considerable differences occurred in the relative yields of plants of com-

parable composition with similar nitrogen fertilization. The sites of the experiments, although in the same field, were well over a ½ mile apart, and while sunshine, temperature, and rainfall were much the same, comparatively high wind velocity was probably partly responsible for relatively limited growth, in case of experiment 29 F 3. (Experimental trials with pineapple plants growing in aliquots of the same soil in asphalt-painted iron containers, furnished with drainage outlets, and abundantly supplied with water and fertilizer, have shown that even moderate wind velocity, if long continued, may decrease the size of plants by as much as 25 per cent as compared with others not exposed to wind but under the same conditions of temperature and sunlight.) Nevertheless, in spite of differences

TABLE 2

EXPERIMENT 29 F 3A: FIELD 5518, PLANTED DECEMBER, 1939. YIELDS, POUNDS PER ACRE OF APPLIED NITROGEN, PERCENTAGE OF LEAF NITRATE-N (NO_3), AND PERCENTAGE OF LEAF AREA EXHIBITING YELLOW-GREEN COLOR (NO. 1), WITH REMAINDER OF LEAF AREA NOS. 2 AND 3 COLORS

PLOT	PRIOR NITROGEN (LB.)	11/18/40		11/19/40	12/14/40		12/30/40		2/14/41	TOTAL NITROGEN (LB.)	FRUIT (TONS PER ACRE)		
		NO_3	No. 1	NITROGEN (LB.)*	DIFFERENTIATION		NO_3	No. 1	(BUDS) No. 1		TOTAL	PLUS OR MINUS D	REQUIRED FOR ODDS 19:1
					NO_3	No. 1							
A....	285	.054	25	60	.070	25	.066	27	15	345	35.54	+2.00	0.64
B....	285	.054	25	80	.073	26	.070	25	15	365	35.40	+1.86	0.64
C....	285	.054	25	100	.074	25	.071	24	12	385	36.33	+2.79	0.64
D....	285	.054	25	None	.064	33	.053	36	30	285	33.54	.....	0.64

* Ammonium sulphate applied in lower leaf axils November 13, but there was not sufficient rain to dissolve and carry it to the roots until November 19.

inherent in the two experimental sites, in both experiments—following diminution in degree of nitrate deficiency with different nitrogen treatments—the yields were increased. When ammonium sulphate was added to all except the D plots, on November 18 (table 2), the plants had a leaf-nitrate content of only 0.054 per cent. This was a low value in relation to the liberal starch reserve indicated by 25 per cent no. 1 leaf color. About a month following fertilization, however, plots A, B, and C increased materially in nitrate as compared with plots D. As a result, the plants of the D plots were outyielded by about 2 tons of fruit per acre. Limited fruit production in the D plots was unquestionably correlated with a nitrate reserve definitely deficient in relation to the high starch content, indicated by over 30 per cent no. 1 leaf color in December. That the plants—especially of the C plots that produced 2.79 tons more fruit per acre than the D—absorbed approximately the maximum amount of nitrate they could metabolize without incurring carbohydrate deficiency is indicated by only 12–15 per cent no. 1 color at budding.

In July, as shown in table 3, the plants in field 5530 were filled nearly to capacity with nitrate (fig. 8) and on the borderline of carbohydrate deficiency, as indicated by only 14 per cent no. 1 color. The decrease in nitrate from 0.097 to 0.070 per cent in only one month, from July to August, might be considered too rapid were it not for the fact that carbohydrate reserves were low, as indicated by slightly less than 15 per cent no. 1 leaf color in both months. These responses were associated, however, with an average 2-hourly temperature for both months of more than 72° but with a light rate of less than 4000 foot candles. Nevertheless, by the end of October, with no nitrogen added to the A plots, leaf nitrate was 0.030 per cent, too low in relation to the abundant carbohydrate reserves indicated by 25 per cent no. 1 leaf color. In contrast, plots B, C, and D, supplied with nitrogen on the dates

TABLE 3

EXPERIMENT 29 F 3: FIELD 5530, PLANTED SEPTEMBER, 1939. YIELDS, POUNDS PER ACRE OF APPLIED NITROGEN, PERCENTAGE OF LEAF NITRATE-N (NO_3), AND PERCENTAGE OF LEAF AREA EXHIBITING YELLOW-GREEN COLOR (NO. 1), WITH REMAINDER OF LEAF AREA NOS. 2 AND 3 COLORS

Plot	Prior nitrogen (lb.)	7/8/40		18/13/40		8/24/40	10/8/40	10/23/40		11/13/40	12/12/40		12/27/40		Total nitrogen (lb.)	Fruit (tons per acre)		
		NO_3	No. 1	NO_3	No. 1	Nitrogen (lb.)	Nitrogen (lb.)	NO_3	No. 1	Nitrogen (lb.)	Differentiation* NO_3	Differentiation* No. 1	NO_3	No. 1		Total	Plus or minus A	Required for odds 19:1
A	260	.097	14	.070	12	None	None	.030	25	None	.039	36	.039	32	260	32.60	.....	0.85
B	260	.097	14	.070	12	80	None	.038	16	80	.062	15	.072	14	420	35.69	+3.09	0.85
C	260	.097	14	.070	12	None	80	.030	24	None	.059	23	.059	19	340	35.15	+2.55	0.85
D	260	.097	14	.070	12	120	None	.045	11	None	.054	18	.060	16	380	35.69	+3.09	0.85

* Budding occurred about the middle of February.

and in the amounts given in table 3, were in December materially higher in nitrate than the A plots, were lower in carbohydrates, and outyielded the nitrate-deficient A plots by about 3 tons of fruit per acre. The second application of nitrogen to the B plots did not increase yields over the D. While the difference in total amount of nitrogen added to B and D plots was only 40 pounds, the second fertilization of the B plots was made when the plants exhibited only 16 per cent no. 1 leaf color, and—while nitrate was subsequently nearly doubled—carbohydrates were continually at a very low level, for no. 1 color remained at 14–15 per cent. It is highly probable that lack of carbohydrates limited reduction of nitrate and new protein synthesis.

The results reported in table 4 show that on November 18 the plants in field 5530 contained only about half the maximum possible nitrate content (fig. 8). Nevertheless, 0.054 per cent leaf nitrate was clearly adequate, for carbohydrates associated with the climatic conditions already noted were low, as indicated by

16 per cent no. 1 leaf color. If past weather records (usually indicative of comparatively favorable conditions for carbohydrate accumulation in field 5530) are considered, and the fact that there were yet about 4 months before budding—during

TABLE 4

EXPERIMENT 29 F 3A: FIELD 5530, PLANTED SEPTEMBER, 1939. YIELDS, POUNDS PER ACRE OF APPLIED NITROGEN, PERCENTAGE OF LEAF NITRATE-N (NO_3), AND PERCENTAGE OF LEAF AREA EXHIBITING YELLOW-GREEN COLOR (NO. 1), WITH REMAINDER OF LEAF AREA NOS. 2 AND 3 COLORS

PLOT	PRIOR NITROGEN (LB.)	11/18/40		11/19/40	12/13/40		12/26/40		2/14/41	TOTAL NITROGEN (LB.)	FRUIT (TONS PER ACRE)		
		NO_3	No. 1	NITROGEN (LB.)*	DIFFERENTIATION		NO_3	No. 1	BUD No. 1		TOTAL	PLUS OR MINUS D	REQUIRED FOR ODDS 19:1
					NO_3	No. 1							
A....	340	.054	16	60	.063	14	.073	18	21	400	35.55	+0.92	0.62
B....	340	.054	16	80	.071	20	.086	16	17	420	35.86	+1.23	0.62
C....	340	.054	16	100	.063	18	.079	19	22	440	36.25	+1.62	0.62
D....	340	.054	16	None	.054	23	.056	22	35	340	34.63	.....	0.62

* Ammonium sulphate applied in lower leaf axils on November 13, but there was not sufficient rain to dissolve and carry it to the roots until November 19.

TABLE 5

EXPERIMENT 29 F 1: FIELD 5510, PLANTED JANUARY, 1939. YIELDS, POUNDS PER ACRE OF APPLIED NITROGEN, PERCENTAGE OF LEAF NITRATE-N (NO_3), AND PERCENTAGE OF LEAF AREA EXHIBITING YELLOW-GREEN COLOR (NO. 1), WITH REMAINDER OF LEAF AREA NOS. 2 AND 3 COLORS

PLOT	PRIOR NITROGEN	8/28/39		9/15/39	10/7/39		11/27/39		12/25/39	1/4/40		2/12/40		TOTAL NITROGEN (LB.)	FRUIT (TONS PER ACRE)		
		NO_3	No. 1	NITROGEN (LB.)	NO_3	No. 1	PRE-DIFFERENTIATION		NITROGEN (LB.)*	NO_3	No. 1	BUDS			TOTAL	PLUS OR MINUS D	REQUIRED FOR ODDS 19:1
							NO_3	No. 1				NO_3	No. 1				
A....	285	.102	17	80	.091	24	.086	21	None	.071	19	.045	17	365	34.67	+0.63	0.92
B....	285	.102	17	None	.087	25	.071	30	80	.058	25	.056	16	365	34.74	+0.70	0.92
C....	285	.102	17	80	.091	24	.086	21	80	.074	19	.055	13	445	36.29	+2.25	0.92
D....	285	.102	17	None	.087	25	.071	30	None	.058	28	.039	28	285	34.04	.....	0.92

* Ammonium sulphate applied in lower leaf axils on December 6, but there was not sufficient rain to dissolve and carry it to the roots until the last of the month.

which period nitrate might become deficient—additional application of nitrogen would be required, particularly since analyses prior to those of November 18 showed that nitrate was decreasing rapidly. Following application of nitrogen to plots A, B, and C, there was (table 4) an increase in leaf nitrate that by December 26 was nearly up to maximum for the stage of growth (fig. 8). This was coupled

with maintenance of adequate starch reserves, as indicated by more than 15 per cent no. 1 color in all plots by the end of December and at budding in February. Associated with these responses was a light rate from December through February of more than 5000 foot candles. Correlated with little or no deficiency of either nitrate or carbohydrates in plots A, B, and C, the plants significantly outyielded those of the D group which were clearly deficient in nitrate in relation to their comparatively high starch reserves.

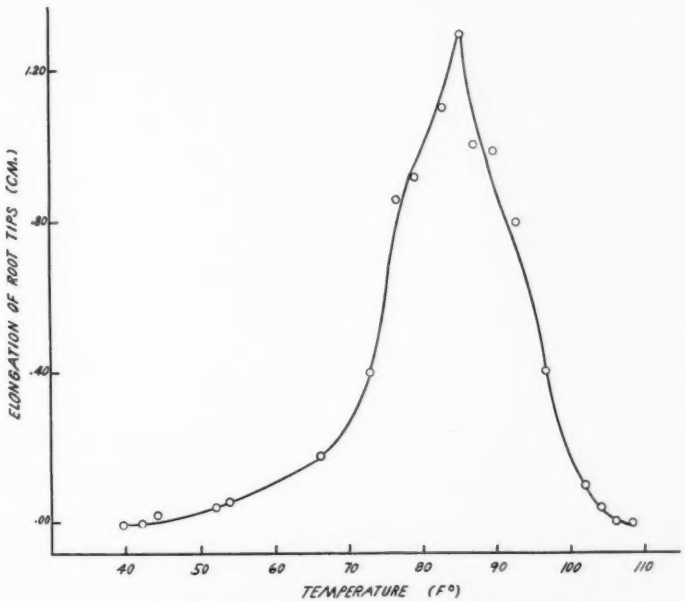

FIG. 9.—Comparative elongation of pineapple roots in tap water as affected by temperature. Variety Smooth Cayenne. Data from WATANABE (30). Roots of standard length measured before and after 48 hours at temperatures indicated. Note limited growth below 68° and above 98°.

The foregoing report on relative yields associated with various degrees of nitrate deficiency, in plants containing sufficient reserves of carbohydrates, is characteristic of an enormous amount of experimental evidence obtained in many locations during a period of several years. There would seem to be little gained, however, by mere repetition of similar plant performances in other experiments. But table 5 includes, in addition to results similar to those already discussed, rather clear indications of the importance of continually maintaining adequate nitrate reserve in relation to carbohydrates the last 3 months before buds emerge. This is in contrast to earlier stages of growth when, as will be shown, plants may be per-

mitted to decrease materially in nitrate in relation to carbohydrates for 1–2 months with little or no effect on yields. Table 5, however, shows that this is far from the

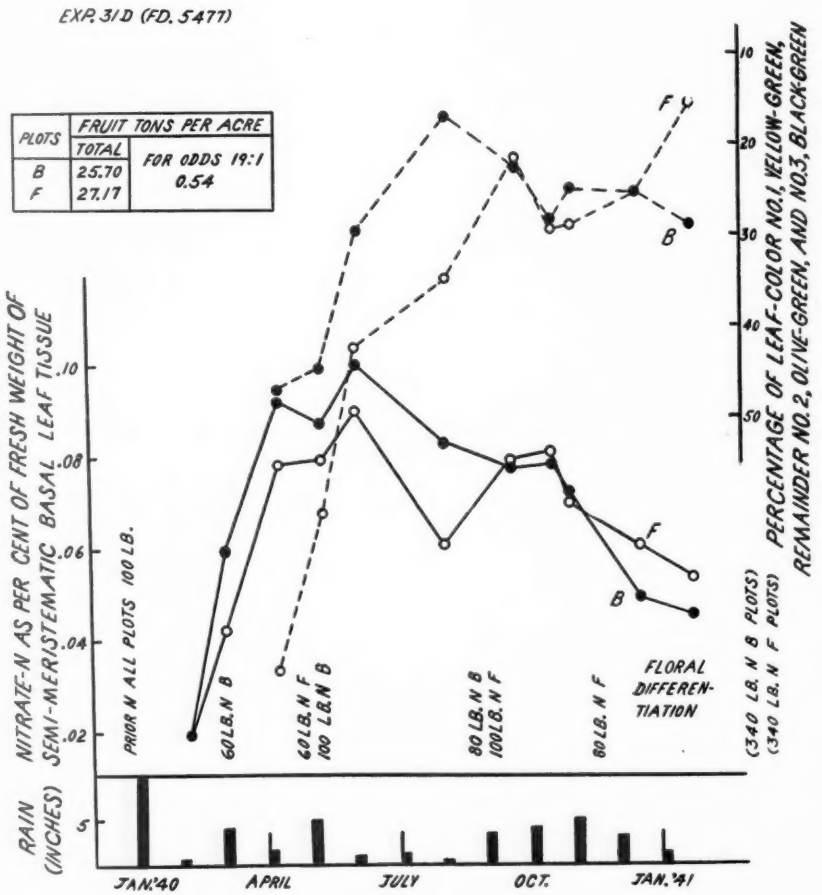

FIG. 10.*—With same total nitrogen fertilization, plots F outyielded plots B, demonstrating relative importance at floral differentiation of adequate nitrate in relation to no. 1 leaf color. Compare earlier months when plants of F series were nitrate deficient and those of B were not. At differentiation, plants of B were too low in nitrate for the high carbohydrate reserve indicated by 30 per cent no. 1 color.

* Figs. 10–14: broken lines, leaf color; solid lines, leaf nitrate.

case during the period just prior to budding, for the C plots—continually maintained with adequate nitrate reserves—gave a gain of over 2 tons of fruit per acre as compared with the D plants, which were extremely deficient in nitrate. This

was true even though prior to October, at initiation of the experiment, all the plants were filled to capacity (fig. 8) with nitrate, the leaf value being 0.102 per cent coupled with but 17 per cent no. 1 leaf color. In contrast to the high-yielding

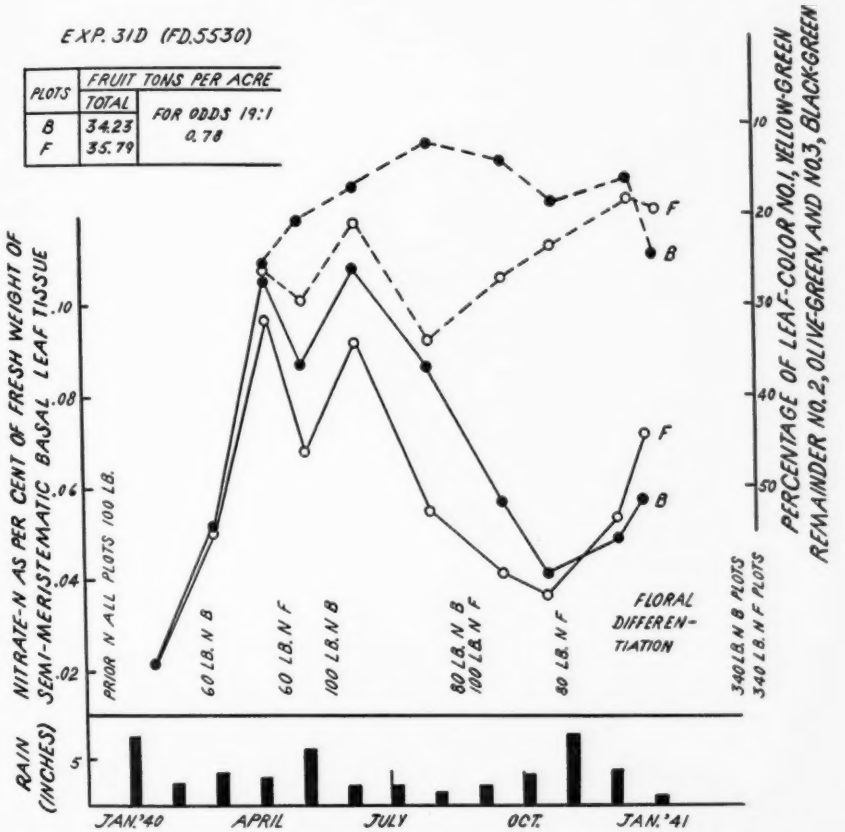

FIG. 11.—As in fig. 10, note relative importance at floral differentiation of avoiding nitrate deficiency in relation to carbohydrates (no. 1 color). Plots F, adequately supplied with nitrate at this period, outyielded those of B that in relation to carbohydrates (no. 1 color) were deficient in nitrate at that time but highest in nitrate earlier. Note much higher nitrate reserves at differentiation than in field 5477 (fig. 10). Field 5530 is a relatively warm location. Greater nitrate absorption but carbohydrate deficiency did not occur, as even F plots were over 15 per cent no. 1 color at differentiation.

C plots, the plants of the A plots were deficient in nitrate in January and February, and the B plants were lacking in nitrate from November through December and at least the early part of January. The temporary elimination of nitrate deficiency in

the A and B plots by the application of 80 pounds of nitrogen, as indicated in table 5, did not give significant increases in yield. On the other hand, the C plots,

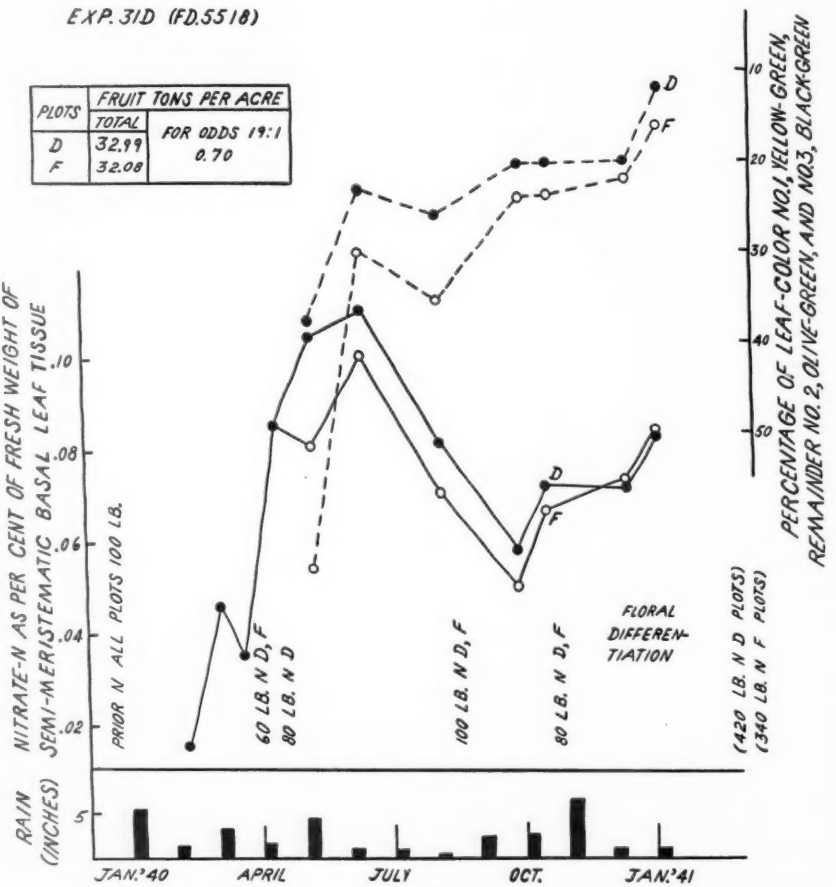

FIG. 12.—In contrast to figs. 10 and 11, at floral differentiation nitrate was not deficient in relation to carbohydrates in either plots D or F, for they approached the arbitrary point of carbohydrate deficiency, about 15 per cent no. 1 color. Earlier, F was deficient in nitrate in relation to no. 1 color. Accordingly F was significantly but not as greatly outyielded by D as would probably have been the case if nitrate had been low at differentiation instead of earlier.

that at no time were deficient in nitrate owing to the 160 pounds of nitrogen added, gave more than three times the gain of either A or B.

The time element was undoubtedly important in relation to these responses. As

nitrate is not directly essential for the growth of pineapple (**24, 25**) or probably any other green flowering plant (**17, 18**), it may well be asked why it is necessary to maintain any particular concentration of it in the pineapple plant as long as the

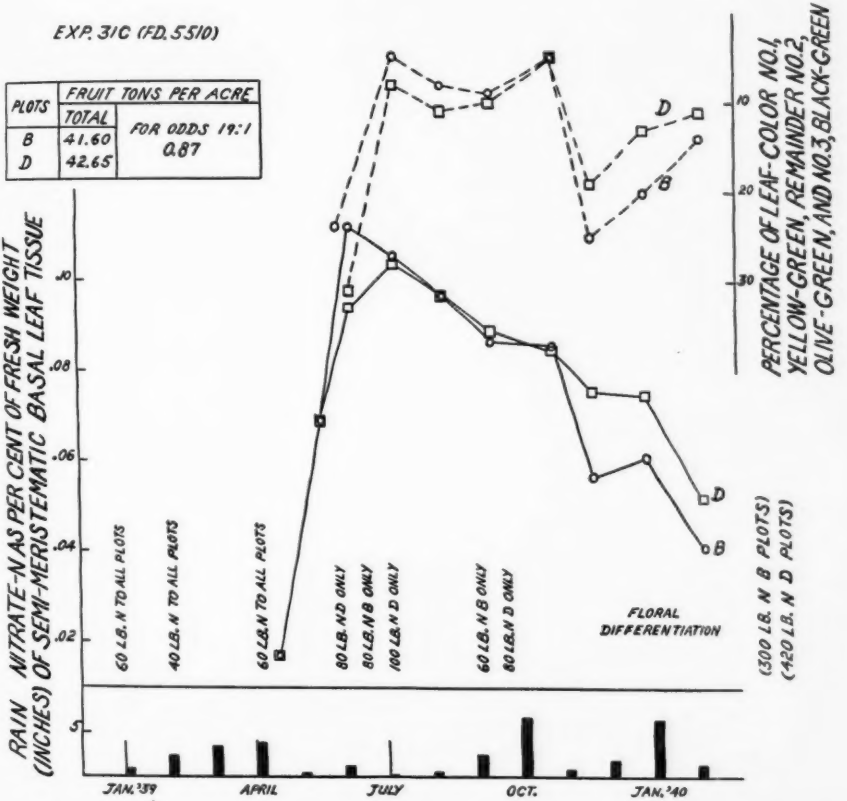

FIG. 13.—Unlike figs. 10–12, note that prior to floral differentiation (not at differentiation) plots D and B were about equally and adequately supplied with nitrate. Higher nitrate in midsummer could not have increased yields, no. 1 color being only 10 per cent, indicative of approaching carbohydrate deficiency. With higher carbohydrate reserves indicated in November by increase to about 20 per cent no. 1 color, B should have heen higher in nitrate. It was accordingly outyielded by D, that in relation to carbohydrates was adequately supplied with nitrate.

reserve of nitrate is not completely exhausted. While the answer cannot be given, it would seem that nitrate in the plants at low concentration is not metabolized as efficiently, or freely, per unit of stored nitrate as when the concentration is higher, provided sufficient carbohydrates are available for the growth responses desired

and for oxidation and energy, as nitrate is reduced. In other plants that accumulate nitrate, as tomato, if the external source of nitrogen is chiefly nitrate, as it probably is in most tillable agricultural soils, there must continually be maintained

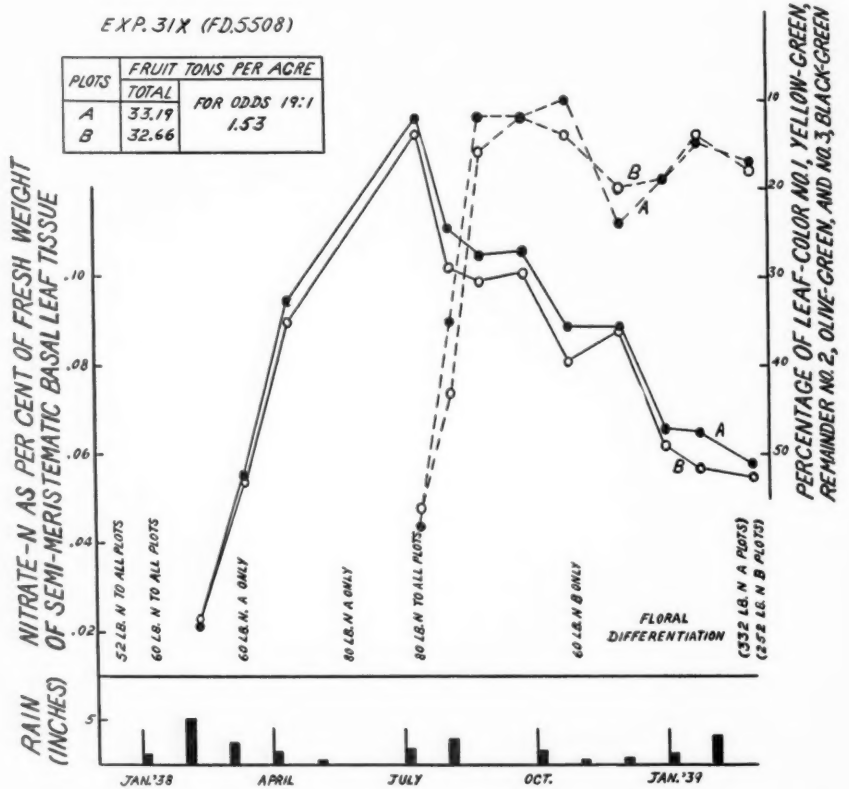

FIG. 14.—Prior to July, plants of plots A and B were filled to capacity with nitrate. Nitrate dropped after July, but in neither treatment became deficient in relation to carbohydrates, for no. 1 color after July was about 15 per cent or lower, indicative of low carbohydrate reserves. Accordingly, heavier nitrogen applications to A than to B were ineffective. Tables 1–6 give positive effects of nitrogen added to high-carbohydrate plants; tables 7–10 give additional examples of negative effects of nitrogen added to low-carbohydrate plants.

in the plant a fairly high concentration of nitrate to insure a synthesis from it of organic nitrogen sufficiently rapid to permit vigorous growth (18). Rapid growth and protein synthesis from nitrate practically cease in tomato, and apparently in pineapple, considerably before the nitrate reserves are completely exhausted.

The C plots, as shown in table 5, were not deficient in nitrate at any time during the experimental period. It does not seem likely, however, that they received an excess of nitrogen, for at the time the buds became evident they were slightly below the arbitrary point of 15 per cent no. 1 leaf color—at which pineapple plants are considered to be deficient in starch reserves. Certainly, prior to the initiation of experiment 29 F 1 in field 5510, there was no over-fertilization with nitrogen, as indicated in June, for instance, when routine field records for the proximate site of the experimental area showed that leaf nitrate was 0.088 per cent, with 38 per cent no. 1 leaf color. A nitrate value under these circumstances of 0.100 per cent

TABLE 6

EXPERIMENT 29 F 3A: FIELD 5403, PLANTED OCTOBER, 1939. YIELDS, POUNDS PER ACRE OF APPLIED NITROGEN, PERCENTAGE OF LEAF NITRATE-N (NO_3), AND PERCENTAGE OF LEAF AREA EXHIBITING YELLOW-GREEN COLOR (NO. 1), WITH REMAINDER OF LEAF AREA NOS. 2 AND 3 COLORS

Plot	Prior nitrogen (lb.)	10/18/40		11/19/40	12/12/40		12/26/40		2/14/41	Total nitrogen (lb.)	Fruit (tons per acre)		
		NO_3	No. 1	Nitrogen (lb.)*	Differentiation		NO_3	No. 1	Buds No. 1		Total	Plus or minus D	Required for odds 19:1
					NO_3	No. 1							
A....	340	.032	21	60	.040	19	.052	12	22	400	33.77	+2.28	1.07
B....	340	.032	21	80	.039	23	.053	13	21	420	33.99	+2.50	1.07
C....	340	.032	21	100	.041	21	.056	14	19	440	34.63	+3.14	1.07
D....	340	.032	21	None	.028	31	.030	19	32	340	31.49	.....	1.07

* Ammonium sulphate applied in lower leaf axils November 9, but there was not sufficient rain to dissolve and carry it to the roots until November 19.

would not have been too high (fig. 8), but that the C plots received practically ideal nitrogen nutrition after initiation of the experiment, and that this was correlated with marked, significant increases in yields, has been noted. Obviously, not less than about 445 pounds of nitrogen per acre was needed for maximum fruit production in field 5510 under the very favorable climatic conditions that prevailed. This is in contrast to results to be reported for the crop a year earlier in field 5508 (fig. 14). The two fields adjoin each other and yet, while about 445 pounds of nitrogen per acre was required for the 1940 crop in field 5510, only about 252 pounds of nitrogen was needed for the 1939 crop, a difference of more than 75 per cent in nitrogen requirements in successive years in essentially the same location.

The following data pertain to the responses of nitrate-deficient pineapple plants under conditions of comparatively low temperature. In some respects the results for field 5403, typical of other cool locations (table 6), seem aberrant in relation

to the responses of other species, since low temperature apparently is not conducive to carbohydrate accumulation in pineapple. It is well known for many types of plants that moderately low temperatures, although not favoring rapid carbon-dioxide assimilation, tend to conserve carbohydrates already contained, as well as those newly synthesized. This is probably owing mainly to a decrease in rate of respiration of sugars or their derivatives (**18**). In field 5403 the average 2-hourly air temperatures were—as usual—relatively low, being respectively about 67°, 65°, and 63° for December, January, and February of the experimental period at the nearest weather station, about a mile away. Actually the air temperatures at the site of the experiment were probably 1°–2° lower, as judged from occasional temperature readings. The light rate for the same months at the nearest weather station was about 5000 foot candles. Considering their low nitrate content at the initiation of the experiment (table 6), however, the plants were not very high in carbohydrates, as indicated by only 21 per cent no. 1 leaf color.

Another notable feature is that at time of differentiation, although white root tips were abundant and soil moisture adequate, leaf nitrate had been raised from 0.032 to only 0.041 per cent by 100 pounds of nitrogen added about a month before. This figure is in contrast to 0.075–0.086 per cent at differentiation, which, when coupled with adequate carbohydrate reserves, has been shown to be essential for maximum production of fruit (tables 1–5). But that such high nitrate values, if practically attainable, in field 5403 would probably not have increased yields but would have been associated with extreme carbohydrate deficiency, is indicated by the low percentage of no. 1 color (12–14 per cent) in the nitrogen-supplied plants of plots A, B, and C. The plants displayed this leaf color on December 26, even though nitrate at that time was 0.056 per cent, which may be compared with a maximum possible value of about 0.085 per cent (fig. 8). There was seemingly a low rate of absorption of nitrate; on the other hand, with the considerable increases in yields associated with increased nitrate reserves (table 6), it does not seem likely that reduction of nitrate and new protein synthesis that occurs mainly in the leaves (**24, 25**) could have been seriously limited by low air temperature. What influences the low air temperatures concerned may have had upon carbon-dioxide exchange is problematical. It is significant that the plants of plots A, B, and C, although low in actual percentage of nitrate, for the most part contained enough, in relation to carbohydrates, as indicated by leaf-color values. Although the plants in field 5403 were on an apparently comparatively low plane of nitrate and CO_2 nutrition, the condition of balance noted was correlated with increases in yields of 2–3 tons of fruit per acre as compared with the plants of the D plots that exhibited a condition of imbalance owing to nitrate deficiency (table 6).

SOIL TEMPERATURE AND NITRATE ABSORPTION.—Because carbohydrate reserves were low and large increases in nitrate would therefore have been unde-

sirable, the apparently slow rate of absorption of nitrate by the plants in field 5403 probably did not influence yields unfavorably, although if absorption had occurred more freely, lesser amounts of fertilizer might well have been employed. But if opportunity for carbohydrate accumulation, in this or another field, should at any time be much greater, then any factor that materially repressed absorption of nitrate would be decidedly disadvantageous. There may be several reasons for this limited uptake of nitrate. While certain desirable data are not at hand, SCHROEDER (**23**) found that cucumber roots absorbed practically no water at 55° and that absorption was about twice as rapid at 70° as at 60°. Similar information for pineapple does not appear to be available, but WATANABE (**30**) has shown that until a temperature of about 68°–70° is reached, pineapple root tips elongate very slowly (fig. 9). It does not seem unlikely that the same temperatures that limit growth of roots would also repress other activities, including absorption of nitrate. While continuous records of soil temperature were not obtained and momentary readings taken during the day at monthly intervals (at a depth of about 8 inches between the plants at the edge of the mulch paper) leave much to be desired, determinations show that the soil temperatures in field 5403 on December 12 and 26, respectively, in the area of the experiment, were 69° and 67°. These temperatures may be compared with determinations in field 5510. There nitrate was apparently freely absorbed (table 5), and on the most nearly corresponding dates the soil temperatures were 73.4° and 72.5°. At least, when they were read, the soil temperatures in field 5403 were just about at the point where there is a sharp break in the curve for rate of root growth (fig. 9). A slight increase or decrease in temperature would probably affect the rate of growth and possibly the absorption of nitrate.

As already pointed out, the percentage of white root tips was comparatively high in field 5403. This evidence is not, however, contradictory to WATANABE'S (**30**) results (fig. 9). A high percentage of meristematic or semi-meristematic white root tip tissue does not always indicate that the roots are increasing in volume or elongating rapidly. It is just as indicative of a slow rate of maturation and of differentiation of secondary elements, including lignified xylem vessels, fibers, and suberized cells. This situation was exhibited by peach and apple roots that remained largely undifferentiated for weeks at 55° and yet increased in length very slowly. At 75°, elongation of roots was rapid, but so also was differentiation, and at no time was there as great a proportion of white root tips as at lower temperatures (**16**).

CARBOHYDRATE DEFICIENCY AT TIME OF FLORAL DIFFERENTIATION.—The striking increases in yields which occurred when nitrate deficiency was corrected by applications of nitrogen during the period of differentiation and development of flower buds have been noted. The results presented in tables 7–10 show that—during the same stage of growth—increases in concentration of nitrate in the pineapple

plant, when coupled with carbohydrate deficiency (as arbitrarily indicated by less than 15 per cent no. 1 leaf color), typically did not increase yields significantly and were sometimes associated with apparent decrease in production of fruit. In field 5426 the plants of all the plots were approximately filled to capacity with nitrate by the end of September (table 7). The plants of the B plots contained nearly the maximum possible nitrate reserve (fig. 8) and were on the borderline of carbohydrate deficiency, as they displayed only 14 per cent no. 1 leaf color in September, even though up to that time they had been provided with less than 200 pounds of nitrogen per acre in contrast to over 300 for plots A and AX.

The B plots were fertilized, however, with the objective of obtaining the maximum possible yields on the site of the experimental area without employing a

TABLE 7

EXPERIMENT 31 X: FIELD 5426, PLANTED NOVEMBER, 1937. YIELDS, POUNDS PER ACRE OF APPLIED NITROGEN, PERCENTAGE OF LEAF NITRATE-N (NO_3), AND PERCENTAGE OF LEAF AREA EXHIBITING YELLOW-GREEN COLOR (NO. 1), WITH REMAINDER OF LEAF AREA NOS. 2 AND 3 COLORS

PLOT	PRIOR NITROGEN (LB.)	9/23/38		9/25/38	10/25/38		10/29/38	12/1/38		12/29/38		1/25/39		3/1/39		TOTAL NITROGEN (LB.)	FRUIT (TONS PER ACRE)		
		NO_3	No. 1	NITROGEN (LB.)*	NO_3	No. 1	NITROGEN (LB.)	NO_3	No. 1	DIFFERENTIATION NO_3	DIFFERENTIATION No. 1	NO_3	No. 1	BUDS NO_3	BUDS No. 1		TOTAL	PLUS OR MINUS B	SIGNIFICANCE
A.....	332	.105	6	None	.090	20	None	.079	26	.048	34	.058	23	.058	19	332	30.3	−0.70	None
B.....	192	.096	14	None	.080	21	60	.084	20	.062	22	.060	17	.058	22	252	31.0	.....	None
AX.....	352	.096	10	80	.084	18	None	.084	19	.057	24	.064	18	.061	19	432	30.6	−0.40	None

* Ammonium sulphate applied in lower leaf axils September 9, but there was not sufficient rain to dissolve and carry it to the roots until September 25.

luxury supply of nitrogen. Consequently the plants were not supplied with additional nitrogen until they had built up a higher carbohydrate reserve, as indicated by an increase from 14 to 21 per cent no. 1 leaf color at the end of October. Nitrogen was then applied sparingly, employing only a 60-pound application because carbohydrate reserves were not higher. The B plots were at least not outyielded by the others receiving up to 160 pounds more nitrogen per acre (table 7). That the application of 80 pounds of nitrogen to the AX plots in September would be futile might be anticipated, for the limiting factor was carbohydrates and clearly not nitrate. The plants were filled nearly to capacity with it, but were definitely deficient in starch, no. 1 leaf color being only 10 per cent. Subsequently the plants of the AX plots built up a slightly higher carbohydrate reserve, but not sufficient to require a nitrogen application, for nitrate reserves remained high in relation to carbohydrates.

The A plots present an interesting situation in that, although supplied with a luxury amount of nitrogen, the timing of the applications of the different increments of it was so poor that at about the time of differentiation still more nitrogen was needed, for the plants had decreased to 0.048 per cent nitrate, too low (tables 1–6) for 34 per cent no. 1 leaf color with which it was coupled. A light application of about 60 pounds of nitrogen to the A plots at about time of differentiation might therefore have increased yields slightly, provided the fertilizer became immediately available.

Field 5518, when nitrate deficiency was corrected (table 1), showed striking increases in yield, up to 3.74 tons per acre. These favorable responses followed the application of nitrogen to plants that exhibited 21 per cent no. 1 leaf color. It is a

TABLE 8

EXPERIMENT 31 D: FIELD 5518, PLANTED NOVEMBER, 1939. YIELDS, POUNDS PER ACRE OF APPLIED NITROGEN, PERCENTAGE OF LEAF NITRATE-N (NO_3), AND PERCENTAGE OF LEAF AREA EXHIBITING YELLOW-GREEN COLOR (NO. 1), WITH REMAINDER OF LEAF AREA NOS. 2 AND 3 COLORS

Plot	Prior nitrogen (lb.)	9/24/40		10/21/40		10/23/40	12/11/40		12/27/40*		Total nitrogen (lb.)	Fruit (tons per acre)		
		NO_3	No 1	NO_3	No. 1	Nitrogen (lb.)	NO_3	No. 1	Differentiation			Total	Plus or minus B	Significance
									NO_3	No. 1				
B.....	340	.059	19	.073	18	None	.062	22	.068	20	340	32.43	.....	None
D.....	340	.060	21	.072	21	80	.072	20	.085	13	420	32.99	+0.56	None

* Budding in late February, 1941.

coincidence that the plants described in table 8, likewise in field 5518, just prior to a nitrogen application, also displayed 21 per cent no. 1 color. In contrast, however, they gave no significant increases in yield from added nitrogen, even though leaf nitrate was ultimately greatly increased. The answer to these seemingly divergent responses is not apparent until the relative nitrate reserves are considered, not only for the respective dates just prior to nitrogen treatments but in each case at an earlier date of analysis. As shown in the first case (table 1), leaf nitrate decreased rapidly in one month from 0.090 to 0.063 per cent, the latter figure being in combination with 21 per cent no. 1 color. In the second instance, where no significant increases in yield followed fertilization (table 8), there had been just prior to the nitrogen application an increase in nitrate, in a little less than one month, from 0.060 to 0.072 per cent, with no. 1 color remaining constant at 21 per cent. Evidently the addition of nitrogen to plants displaying this color, indicative of a rather low starch reserve, would seldom be considered advisable with nitrate in-

creasing rapidly and at the concentrations recorded. Eighty pounds of nitrogen, added to the plants of the D plots, increased nitrate to approximately the maximum possible level at differentiation, and there was a gain in yield—not significant—of only 0.56 of a ton per acre. But the high nitrate value was associated with low concentration of carbohydrates, for the plants exhibited only 13 per cent no. 1 leaf color. Unquestionably carbohydrates, and not nitrate, were the limiting factor to greater tonnage.

This discussion is concerned with the condition of the plant during the last 3–4 months before buds appeared, not particularly with the agricultural practices employed, with prior fertilizer treatments, or with the fluctuating array of environmental influences characteristic only of the season and of a specific site. On the other hand, the responses of plants, quantitatively described, furnish informa-

TABLE 9

EXPERIMENT 31 C: FIELD 5415, PLANTED NOVEMBER, 1938. YIELDS, POUNDS PER ACRE OF APPLIED NITROGEN, PERCENTAGE OF LEAF NITRATE-N (NO_3), AND PERCENTAGE OF LEAF AREA EXHIBITING YELLOW-GREEN COLOR (NO. 1), WITH REMAINDER OF LEAF AREA NOS. 2 AND 3 COLORS

PLOT	PRIOR NITROGEN (LB.)	8/16/39		9/12/39		9/21/39	10/20/39		11/21/39			12/26/39		2/6/40		TOTAL NITROGEN (LB.)	FRUIT (TONS PER ACRE)		
		NO_3	NO. 1	NO_3	NO. 1	NITROGEN (LB.)	NO_3	NO. 1	PRE-DIFFERENTIATION			NO_3	NO. 1	NO_3	NO. 1		TOTAL	PLUS OR MINUS D	SIGNIFICANCE
									NO_3	NO. 1									
B....	360	.096	15	.084	12	None	.067	17	.062	31		.050	24	.039	23	360	38.03	+0.15	None
D...	340	.089	18	.072	14	80	.087	16	.068	28		.054	20	.043	16	420	37.88	.....	None

tion that is of general use in subsequent years even though a vast number of different environmental patterns and fertilizer treatments may lead to the production of plants essentially similar in content of nitrate and carbohydrates. Thus, in table 9 it is not implied that either of the plots had an efficient schedule of nitrogen applications prior to August, although the nitrogen nutrition of the plants in earlier months of growth is discussed elsewhere. The fact is simply emphasized that the plants of the D plots, even though decreasing in nitrate, were bordering on carbohydrate deficiency shortly before they received 80 pounds of nitrogen on September 21. That this application of nitrogen materially increased the nitrate content a month later is apparent from table 9. Nevertheless, this was not correlated with any increase in tonnage, but rather with an apparent but not statistically significant loss. Negative effects from nitrogen added to plants low in carbohydrates is a characteristic response that has occurred repeatedly. The plants of the D plots thus responded typically, for just before and a month after nitrogen was applied they displayed, respectively, only 14 and 16 per cent of no. 1 leaf color—indicative

of low carbohydrate reserves. Further, nitrogen added when plants are deficient in carbohydrates frequently appears to be a total loss, even though subsequently there may be accumulated a more liberal carbohydrate supply, as in the plants of the D plots just before differentiation. These plants at that time might have given increased yields if nitrate had then been higher in relation to the prevailing leaf color, 28 per cent no. 1. On October 24, however, 4.2 inches of rain fell, and during the month there was nearly 7 inches. This may have resulted in removal of nitrate, in whole or in part, from the soil in the vicinity of the root systems.

Much additional evidence might be given to support that already presented. In field 5320 the responses were sufficiently different from the foregoing reports to

TABLE 10

EXPERIMENT 29 F 2: FIELD 5320, PLANTED NOVEMBER, 1938. YIELDS, POUNDS PER ACRE OF APPLIED NITROGEN, PERCENTAGE OF LEAF NITRATE-N (NO_3), AND PERCENTAGE OF LEAF AREA EXHIBITING YELLOW-GREEN COLOR (NO. 1), WITH REMAINDER OF LEAF AREA NOS. 2 AND 3 COLORS

Plot	Prior nitrogen (lb.)	10/16/39		12/1/39		12/1/39	1/15/40		2/19/40		Total nitrogen (lb.)	Fruit (tons per acre)		
				Pre-differentiation		Nitrogen (lb.)*			Buds			Total	Plus or minus A	Required for odds 19:1
		NO_3	No. 1	NO_3	No. 1		NO_3	No. 1	NO_3	No. 1				
A.....	372	.045	30	.055	22	None	.060	14	.045	14	372	31.92	.....	.....
B.....	372	.045	30	.055	22	80	.074	3	.051	3	452	32.81	+0.89	0.83

* Ammonium sulphate applied in lower leaf axils on November 16, 1939, but there was not sufficient rain to dissolve and carry it to the roots until after December 1, 1939.

make brief mention of them worth while. Before differential nitrogen treatments were introduced (table 10), the plants exhibited 30 per cent no. 1 leaf color and only 0.045 per cent leaf nitrate-nitrogen. The maximum possible nitrate content for this stage of growth is about 0.100 per cent (fig. 8). Knowing nothing about the climatic conditions characteristic of field 5320, the conclusion might be reached that these plants were definitely deficient in nitrate in relation to carbohydrates, as 30 per cent no. 1 color is indicative of a very liberal reserve of starch. While such was presumably the case, the plant condition that prevailed would not necessarily indicate that substantial increases in yields would occur following increase in nitrate reserves. In the first place, it should be noted that—without any ammonium sulphate being added—leaf nitrate increased during November from 0.045 to 0.055 per cent, and no. 1 leaf color during the same period decreased from 30 to 22 per cent. Soil nitrate fluctuates materially (2) and may have been in part responsible for the rise in leaf nitrate. But even more critical is the fact that the

light rate in fall and winter is typically low in the area of field 5320. For example, during the experimental period for November–January, the light rate was almost exactly 3500 foot candles each month. These conditions are not favorable for abundant accumulation of carbohydrates, particularly when—with about 16,000 plants per acre—the lower leaves are exposed to but a small fraction of the total light intensity. Correlated with the low light rate, even the A plots (to which no nitrogen was added during the experimental period) were just on the arbitrary borderline of carbohydrate deficiency in January and February, for they displayed only 14 per cent no. 1 leaf color. On the other hand, the B plots, to which 80 pounds of nitrogen was added the first of December, increased in leaf nitrate over A, but this was correlated with a decrease in no. 1 leaf color to 3 per cent. Associated with these responses was an increase of less than 1 ton of fruit per acre, just within the limits of statistical significance.

The foregoing experiments, with many similar ones not here reported, furnish a fair picture of the high degree of precision that can be attained in the nitrogen nutrition of pineapple. With plant records taken in routine field analyses at monthly intervals, however, and with weather for the succeeding month uncertain, absolute precision in fertilization is unattainable. Thus, if sunshine and temperature for any particular location were much more favorable for carbohydrate accumulation than would be anticipated from weather reports in other years, increased nitrate reserves might well be associated with gains in yields that would not usually occur. On the other hand, if carbohydrate accumulation were limited by unusually adverse weather conditions, gains in fruit tonnage from increases in the nitrate level would be lacking, or much less than anticipated.

As will be shown presently, extreme precision in the timing of nitrogen applications is apparently unnecessary in the earlier stages of growth. The foregoing remarks apply particularly, therefore, to the last 3 months before budding. But if plant records are taken as described and employed as a guide to nitrogen requirements, a contemplated application of nitrogen may usually be depended upon to produce certain definite results, even though the weather may be somewhat atypical for the site, except where plants are on the borderline of nitrate or carbohydrate deficiency. And under either of these conditions, a not very great shift in weather might materially influence the results obtained.

This report of investigations on only fruitful classes of pineapple plants, all within the range of types that for convenience KRAUS and KRAYBILL (**10**) placed in a single class, is strongly supported by the studies which RUSSELL and BISHOP (**22**) have made of barley, wherein they likewise employed only fruitful plants growing under field conditions within a relatively narrow range of practical productivity. After 10 years of research, they state—referring to nitrate and carbohydrate assimilation—"These two factors produce two entirely different effects. Up to a

certain quantity, increases in the nitrate supply in the soil correspondingly increase the nitrate uptake by the plant, and this correspondingly increases its carbohydrate assimilation. The yield thus increases, but the composition of both plant and of grain hardly changes. . . . . Further increases in soil nitrate supply beyond this stage have a different effect: they raise the nitrogen content of the plant as a whole and of the grain, but do not correspondingly increase the yields. There is no sharp point where increased yield ceases and increased nitrogen content begins; both effects overlap, but the change is well marked around a certain nitrogen content." Thus, work with another plant, produced under the climatic conditions prevailing in England, gave results which, in principle, are in entire accord with those of pineapple grown in the semitropical environment of Hawaii. Obviously these principles (10) are not new or peculiar to pineapple, but they are of interest in that they have been directly applicable in increasing the effective use of nitrogen in the nutrition of this plant over very considerable acreages.

NITRATE DEFICIENCY DURING EARLY VEGETATIVE STAGES AND SUBSEQUENT RECOVERY.—It is apparent that the period at which differentiation of flower buds takes place is a critical stage of growth. If at that time nitrate is deficient in relation to carbohydrates, as indicated by plant records, pronounced losses in yield of fruit may be prevented if the deficiency is promptly corrected by addition and absorption of nitrogen. It has also been shown that—even though the concentration of nitrate in the plant may be extremely low—added nitrogen, whether or not it raises the nitrate reserves, may be ineffective if applied to plants deficient in carbohydrates. In contrast, during the earlier stages of growth there is much more latitude. Even when carbohydrate reserves are abundant, as indicated by no. 1 leaf-color values materially higher than 15 per cent, there may be 3–4 months during which nitrate reserves decrease to much lower than the maximum values shown in figure 8, with relatively slight loss in yield of fruit (fig. 12). This is true provided the plants are not deficient in nitrate in relation to carbohydrates during the period of floral differentiation and bud development. A few examples, typical of results that have appeared repeatedly, are given here.

Figure 10 shows that plots B and F both received 340 pounds of nitrogen per acre, but that the plants of the F plots significantly outyielded those of the B plot by 1.47 tons of fruit per acre. This was owing to the fact that the B plots received a predetermined schedule of nitrogen treatment which, within the imposed limit of the 340 pounds, appeared no less desirable than some other that might have been formulated in advance. In contrast, as soon as the plants became established, those of the F plots were fertilized on the basis of the determinations made of leaf nitrate and leaf color, due account also being taken of the absorptive capacity of the root system, as indicated by the percentage of white root tips and the root anchorage. The first differential treatment between the two plots occurred

in March, when the B plots received only 60 pounds of nitrogen. Leaf-color values were not available at that time, because the leaves had not expanded sufficiently to permit taking such records. But with nitrate content increasing rapidly at that time (fig. 10), any immediate application of nitrogen was not essential. In May the B group was again supplied with nitrogen, but this time 100 pounds was employed while the F plots received only 60. Nitrogen was added to the F group because of the flattening off of the nitrate curve and the very high percentage of no. 1 leaf color (fig. 10). More than 60 pounds of nitrogen was not applied, however, because the plants were still very small and the percentage of no. 1 leaf color was decreasing rapidly. One month later the leaf-nitrate content of the B group was about 0.100 per cent, approximately the maximum value attainable (cf. fig. 8). The F plots were slightly lower, 0.090 per cent. In August the plants of the F plots had dropped to 0.060 per cent leaf nitrate and displayed 35 per cent no. 1 leaf color. Additional nitrogen was therefore necessary, and 100 pounds was applied. The B group received 80 pounds. Note the very sharp rise in nitrate and shift from 35 to 20 per cent no. 1 yellow-green color (only in the F plots) about a month after the fertilizer was applied. Note further that the trend of the nitrate and color curves in figure 10 was apparently affected very little, if at all, by the nitrogen added to the B plots. The strong positive response of the F plots to added nitrogen and the seemingly negative reaction of the B groups are characteristic, not only of pineapple but of many other plants. Whatever the reason, it seems consistently true that plants deficient in nitrate and high in carbohydrates—when given a liberal external supply of nitrate—absorb and reduce it much more vigorously per unit of plant weight than do plants with a less abundant carbohydrate reserve and a higher nitrate content (**18**). Thus, during late summer, the plants of the F plots were definitely low in nitrate in relation to carbohydrates, and—as the plant weight records showed—had been growing in volume relatively slowly. They were nevertheless actively storing up a carbohydrate reserve, as indicated by 35 per cent no. 1 color. The time spent in carbohydrate manufacture and therefore not available for vigorous protein synthesis—owing to lack of a sufficient concentration of nitrate in the plant from which freely to manufacture it—was seemingly of little or no disadvantage. When nitrate was again made available, the high carbohydrate plants of the F plots apparently absorbed and reduced it far more actively than they would have, had carbohydrates been materially lower. The B plots, on the other hand, could not efficiently use, and did not need, the nitrogen application at the time they received it; for in August, just before they were provided with 80 pounds, they were on the borderline of carbohydrate deficiency, displaying only about 15 per cent no. 1 leaf color.

Clearly, this nitrogen could later have been used by the plants to advantage if it had remained available in the soil until the B plots, just before floral differentia-

tion, increased in starch reserves to the point where they displayed about 30 per cent no. 1 leaf color. But the nitrogen seemingly was not then available, possibly owing to leaching by fairly heavy rains (fig. 10). The favorable yield given by the F plots is probably owing in large part to the fact that 80 pounds of nitrogen applied in November, as the plants exhibited about 30 per cent no. 1 leaf color, brought them into the critical period of floral differentiation with about the maximum nitrate content that could be carried without incurring carbohydrate deficiency. In February, a little before budding, the plants of the F plots displayed only about 15 per cent no. 1 leaf color.

While perhaps of only incidental interest, it is shown in figure 10 that the leaf-color and leaf-nitrate curves do not always have similar trends. Nitrate reduction accompanied by carbohydrate oxidation, new protein synthesis, and often accelerated respiration (**18**) is only one of several factors that can cause a shift in leaf color and in concentration of carbohydrate reserves. Cloudy weather or shading as the plants become larger and more crowded will often result in darker green color, even if not so extreme as to induce etiolation. Both nitrate determinations and color readings have contributed to increased efficient use of nitrogen in fertilization of pineapple. Neither alone is adequate. For instance, even though nitrate is decreasing rapidly in the F plots from December to February, the plants are becoming greener (fig. 10). On the contrary, during March and April, while the plants of the B plots were approximately filled to capacity with nitrate, they did not decrease significantly in percentage of no. 1 leaf color until the third month, and then continued to increase in greenness for the next 3 months—although nitrate decreased during the same period.

The application of nitrogen just prior to floral differentiation that gave the favorable results shown in figure 10 is no reason for assuming that a similar fertilizer treatment might be effective for the next planting in the same field, as already indicated in tables 7–11. Because climatic conditions vary from year to year, the schedule of nitrogen fertilization cannot be made inflexible, but must vary in accordance with them.

The results shown in figure 11 are essentially similar to those already described for field 5477 (fig. 10), and there is little point in a complete review of the experimental manipulations and plant responses in the relatively warm field 5530. In spite of differences in yields (about 8 tons of fruit per acre between the two locations), owing in large part to unlike environment, the general principles involved are essentially the same. Within the imposed limit of 340 pounds of nitrogen, materially greater yields were obtained in the F plots, which at the time of differentiation contained an adequate nitrate reserve in relation to carbohydrates, even though during the earlier stages of growth they were relatively low in nitrate as compared with the B plots.

As a rule there have been enough experimental plots in any given field so that the various situations presented in figures 10–14 have often been demonstrated in a single location. The principles demonstrated by these responses of the plants are characteristic of the plant itself and not peculiar to an experimental site. Thus, fields 5477 and 5530 both show that if for any reason the nitrogen supply must be limited, it can best be withheld during the earlier stages of growth, not during the period of floral differentiation and development (figs. 10, 11). Yet field 5477, because of comparatively high elevation, has an average 2-hourly temperature for 12 months that is about 4° F. cooler than that of field 5530, a difference that in the aggregate is great and associated with much reduced plant size and yields.

The results shown in figures 10 and 11, and in tables 1–7, demonstrate the importance of maintaining at the time of differentiation an adequate nitrate reserve in relation to carbohydrates. Figure 12 illustrates the responses of two plots, both of which at differentiation were nearly filled to capacity with nitrate for that stage of growth (fig. 8) and on the borderline of carbohydrate deficiency, as indicated by slightly less than 15 per cent no. 1 leaf color. Obviously the plants of both plots were amply supplied with nitrate reserves at that time. Earlier, however, the F plots, which received 80 pounds less nitrogen in April than the D plots, were—except for a brief period in June—definitely deficient in nitrate. This was indicated by the fact that leaf nitrate, in relation to carbohydrate reserves, was much below 0.100 per cent, the proximate maximum value (fig. 8), and the plants exhibited about 30 per cent no. 1 color during most of the summer. In contrast, the plants of the D plots contained approximately as much nitrate as could have been carried without incurring carbohydrate deficiency, for they displayed during most of the summer about 20 per cent no. 1 leaf color, indicative of only moderate carbohydrate reserves. Associated with ample nitrate reserves at time of differentiation and bud development but with a deficiency during earlier stages of growth, as already noted, the F plots were significantly outyielded by only 0.91 tons per acre. This difference does not correspond to the losses in tonnage that often occur when nitrate is deficient in relation to carbohydrates at time of differentiation (tables 1–6).

Figure 13 shows just the reverse situation of that presented in figure 12. The plants of the B plots (fig. 13), deficient in nitrate in relation to carbohydrates only at time of differentiation, were significantly outyielded by the D group, which were continually supplied with adequate nitrate reserves. In fact, both lots of plants probably received during the summer somewhat more nitrate than they could efficiently metabolize, as carbohydrate reserves were then low, no. 1 leaf color being less than 10 per cent. For maximum possible yields, the objective in the D plots, it is obviously essential to keep the plants adequately provided with nitrate in relation to carbohydrates. And the supply of the latter depends in large part

upon the opportunity for carbon-dioxide assimilation. Because this is determined largely by weather conditions that are variable, it follows that an inflexible schedule of nitrogen applications is undesirable. If it should be desirable to limit nitrogen for reasons of cost, it seems certain that this had better be done relatively early, not during the period of floral differentiation or the early stages of bud development.

Figure 14 illustrates another situation, wherein the plants of the A plots received a predetermined schedule of nitrogen fertilization. The B plots were fertilized, employing the usual plant records as guide to nitrogen requirements. For some weeks in the early summer leaf-color readings were omitted. Drought was a limiting factor, as shown by records (not here reported) of severe leaf-water deficiency. Moisture in the white basal semi-meristematic tissue of the leaves was not noticeably depleted, and analyses showed that up to July the plants of both plots were filled to capacity with nitrate. This was true even though up to that time the B plants had received 140 pounds less nitrogen per acre than the A. Following rain in July, with increased plant volume and increased greenness (decrease in percentage of no. 1 leaf color), there was a sharp decline in nitrate reserves. This decrease was undoubtedly owing in large part to utilization of contained nitrate in new protein synthesis at a rate that exceeded nitrate absorption. Accordingly, 80 pounds of nitrogen was applied to the B plots. Following the predetermined plan of fertilization, the A plots were also provided with the same amount. From then on the plants of both plots contained approximately as much nitrate as could be carried without incurring carbohydrate deficiency. During most of the remaining period no. 1 leaf color was only a little above 15 per cent (fig. 14). As might be anticipated, with essentially no nitrate deficiency at any time in either plot, the difference in yield in favor of A of only 0.53 tons of fruit per acre was not significant, though in the former case there was employed 322 and in the latter 252 pounds of nitrogen. In another group of plots, 432 pounds of nitrogen was applied, but the yield did not significantly exceed the B series with only 252 pounds applied.

The experiments upon which this report is based were conducted only in areas where potassium and phosphorus were not limiting factors. Comparable results usually are not obtainable in soils low in potassium or phosphorus, or both. The leaf content of these elements must not be permitted to become deficient. By employing the quantitative records described, the determination and regulation of the nitrogen nutrition of pineapple plants becomes a comparatively precise procedure. Not only has this method placed the nitrogen fertilization of fields on a higher plane of efficiency, but the necessity of distributing large-scale experimental plots over a plantation is not essential. By means of quantitative records an experiment may be initiated at any time, often only 2 months before buds appear, and the effect of withholding or applying nitrogen on yields thus determined. Critical

study of the plant records and associated climatic conditions will show the reasons for gains or losses in yield.

NITRATE AND LOW TEMPERATURE AS CORRELATED WITH FLORAL DIFFERENTIATION.—All the experimental plots described in the present report were in fields planted in the fall and early winter. A year later the plants entered the winter period of low temperatures as nitrate was decreasing (tables 1–10; figs. 10–14). In some sites the plots high in nitrate in relation to carbohydrates fruited 2–3 weeks later than others that contained less nitrate but more liberal carbohydrate reserves. In other locations all the plots differentiated flowers at essentially the same time. This was apparently associated with intimate relationship between nitrate reserves, a single night or several nights of sufficiently low temperature, and the appearance about 5 days later of the earliest stages of floral differentiation recognizable under the microscope.

Similar responses have been recorded by THOMPSON (**28, 29**), PLATENIUS (**20**), KNOTT (**9**), and MILLER (**15**), for celery, onion, spinach, and cauliflower. Low temperatures in the field are often closely correlated with differentiation of flower buds. In the case of cauliflower, ROBBINS (**21**) has reported that nitrate deficiency directly or indirectly resulted in premature flowering. In pineapple, as already noted, both temperature and nitrate reserves appear to play a part in influencing the time of floral differentiation. Whether or not the photoperiod is of significance is not known. The maximum difference in length of day in Hawaii is 2.6 hours. Under suitable nutritional conditions, pineapple plants may differentiate flower primordia under the longest or shortest days that prevail. MCKINNEY and SANDO (**13**) report that "Sexual reproduction in the spring wheats and in the winter wheats is not dependent upon a critical temperature or a critical photoperiod. . . . . However, the time when sexual reproduction occurs is greatly influenced by the temperature and the photoperiod." MELCHERS (**14**) has concluded that certain biennial plants do not blossom unless their growing points have been subjected to low temperatures. It may well be questioned whether it is the growing point or the leaves that are most responsive to effects of low temperature on time of floral differentiation.

The results just cited, and those of ROBBINS (**21**), indicate that both low temperature and low nitrate supply may influence the time of floral differentiation. In the case of pineapple also, these factors may be coupled with initiation of floral primordia. Attempts were made to estimate[2] the date of floral differentiation in

[2] There being an interval of 2 months between floral differentiation and emergence of buds, knowledge of the date of differentiation of a field is of considerable practical value in large-scale agricultural operations, for it furnishes notice of the proximate time of harvest of fruit 2 months earlier than does the date of budding. Of course, dissection of plants to determine whether they show floral differentiation is in general impractical, as it involves destruction of plants and would often have to be continued for weeks before differentiation was finally observable.

field and experimental plots, taking into consideration both the percentage of leaf nitrate-nitrogen and the minimum temperatures. An empirical formula was employed, wherein the percentage of leaf nitrate-nitrogen was multiplied by the minimum temperature on any given night. In general, when this value multiplied by this minimum temperature resulted in an empirical differentiation index figure of about 3.00 or less, the first stages of differentiation recognizable under the microscope occurred about 5 days later. There were frequent exceptions, for differentiation sometimes occurred even though the index figure was materially higher than 3.00, particularly when there were two or three nights of moderately low temperature within a period of 7–10 days prior to differentiation. As yet, no entirely satisfactory method has been found for equating effects of several moderately cool nights occurring consecutively or that lie within any one of several intervals of time, as compared with one cool night, with respect to their influence on the differentiation of plants of known leaf-nitrate values.

Coupled with minimum temperature, the weighted leaf-color values alone, or in conjunction with nitrate content, did not furnish as precise a method of forecasting the time of differentiation as did nitrate content alone. Perhaps this may be related to the fact that the white basal tissue of the leaf employed for nitrate analysis is in close proximity to the meristematic stem tip from which floral primordia arise, or that the nitrate content of the distal end of the stem corresponds closely with that of the white basal tissue of the leaf analyzed. But it seems unlikely that the percentage of nitrate is necessarily correlated directly with floral differentiation, since ammonium may be completely substituted for nitrate as an inorganic nutrient and yet the plants be vigorous and fruitful (**24, 25**).

It is possible that changes in percentage of nitrate accompany quantitative or qualitative changes in the stem tip that induce the synthesis of hormones, compounds which tend toward floral differentiation in conjunction with low temperatures. Even so, it is certain that, at low levels of nitrate supply, differentiation takes place although temperatures are continuously relatively high.

Thus plants comparatively high in nitrate have been found to differentiate floral primordia following a single night of relatively low minimum temperature or several nights of moderately low minimum temperature. On the contrary, plants extremely low in nitrate, or entirely lacking it, often differentiate floral primordia in the hottest months of the year, as when absorption of nitrate is largely prevented by inadequate root systems. Accordingly, in experiments where the plants of different plots are radically different in nitrate reserves, a single night of sufficiently low temperature may be coupled with simultaneous differentiation in all plots. On the other hand, if minimum temperatures are relatively high, the plots with plants low in nitrate often fruit considerably in advance of others that have a higher content.

When fields are planted in the spring, fruiting may frequently be delayed several months by means of heavy fertilization with nitrogen, provided low soil temperatures, or lack of potassium, or other limiting factors do not prevent the free absorption and accumulation of a high nitrate reserve. On the other hand, greatly delayed fruiting typically does not occur in a field planted in the fall. When a field set out in the spring enters the coldest winter months, it is still relatively young, and if there is available a liberal external supply of nitrogen that may be readily absorbed, the plants may contain the highest possible percentage of nitrate, for they are at the stage of growth corresponding to the peak of the curve for leaf nitrate (fig. 8).

Accordingly, unless the spring-planted plants are low in nitrate, the lowest temperature of winter may apparently be without influence on the direct or indirect induction of floral differentiation. As the plants become older, there is correlated with additional months of active growth—but not necessarily with seasonal conditions—a decrease in nitrate that is practically unpreventable. Such a trend is indicated in figure 8. By this time the season of lowest temperature often has passed. And with seasonal temperatures not low enough to play a major role in induction of floral differentiation, directly or indirectly, low nitrate reserves foster early fruiting, while plants higher in nitrate in relation to carbohydrates fruit relatively late. In contrast, fields planted in the fall or early winter are at a stage of growth about a year later when the maximum possible concentration of nitrate is so low (fig. 8) that directly or indirectly a single night of relatively very low temperature, or several nights of moderately low temperature, may apparently induce simultaneous floral differentiation in plots which show variations in nitrate reserves, as indicated in the tables and graphs included in this paper.

As might be anticipated, however, there may be cases in which—because of drought or low air temperatures, or both—growth in the summer or early fall is practically suspended for a period of 2–3 months. Such a field, when it enters the winter season, may still be young in terms of months of growth and at the proximate point of highest possible nitrate content (fig. 8). In such instances, as is true for plantings made in spring, temperatures—even during the winter—are seemingly not low enough directly or indirectly to induce floral differentiation in plants so high in nitrate. Such differentiation may then be delayed, sometimes for nearly a year. If nitrate decreases rapidly enough so that it is low during the warmer months, the time of differentiation may be intimately associated with the prevailing nitrate and carbohydrate reserves in the plants. If nitrate does not decrease materially until the early months of the following winter, then the date of differentiation may approximately coincide with a single night of very low minimum temperature or follow shortly two or three nights in close succession of moderately low minimum temperatures.

Summary

1. Methods are given for obtaining in the field quantitative records of plant weight, number and dimensions of leaves, and percentage deficiency, if any, of stored water in the leaf of the pineapple plant.

2. The potential absorptive capacity of the root system is indicated by records of root anchorage in pounds and percentage of main roots and lateral rootlets displaying white non-suberized tips.

3. Only white, semi-meristematic tissue at the leaf base is analyzed for nitrate, potassium, and phosphorus. Regardless of age in months, prior to blossom-bud development, the leaf employed for analysis is always in precisely the same easily recognized stage of development (fig. 6).

4. After emergence of the determinate blossom cluster, no more analyses for nitrate are made, for the external nitrogen supply after budding is essentially without influence upon the development of the original plant and the fruit it may produce.

5. Until the specialized storage tissue of the leaf becomes practically depleted of water, the white basal tissue does not fluctuate materially in percentage of moisture.

6. Macrochemical analyses for starch, the major carbohydrate reserve expressed as percentage of either green or dry material, completely failed to indicate to what percentage of capacity the plants were supplied with starch. This was owing to the fact that relatively xeromorphic yellow-green plants contained such a high proportion of inert lignified elements that, even though essentially all non-senescent starch-storing parenchymatous tissue was filled to capacity with starch, expression as percentage of the fresh or dry material indicated relatively small amounts of starch. With respect to nitrate nutrition, such a plant is not considered to be deficient in carbohydrates, even though the absolute amount is low.

7. In contrast, black-green, soft, succulent plants containing only about 25 per cent of the starch which their tissues were potentially capable of storing actually had much more starch than the woody ones, whether this content was expressed on the per cubic centimeter basis or as percentage of fresh or dry material. With respect to nitrate nutrition, plants in this category are therefore considered deficient in carbohydrates even though the absolute amount of starch is higher than in the former case.

8. It was determined by means of microchemical observations that the degree of greenness of the leaves reflected what percentage of the total capacity of starch reserves the plant possessed. The greater the degree of yellow-green color of the plant, provided it did not approach senescence, the higher the relative starch content in the sense noted. By employing suitable standards of color (fig. 7), the relative greenness, quantitatively recorded, supplied a sufficiently precise index of

carbohydrate content. Without this index, studies on nitrogen nutrition of pineapple, given in part in this paper, would have been of less significance.

9. It is essential to maintain an adequate reserve of nitrate in the plant. If the concentration of nitrate is relatively low, even though measurable amounts are present, its reduction is not so efficiently or freely effected as when it is higher. Sufficient carbohydrates must be available for oxidation as nitrate is reduced.

10. Plants which had been allowed to accumulate a high carbohydrate reserve and to become temporarily deficient in nitrate, when again supplied with nitrate, apparently absorbed and reduced it much more vigorously than other plants continuously higher in nitrate and lower in carbohydrates. Probably for this reason, provided the time until budding was adequate for plants to recover in content of nitrate and synthesize new protein, there was relatively little loss in yield of fruit, as compared with other plants that were at no time deficient in nitrate relative to carbohydrates.

11. If at any time prior to emergence of flower buds, nitrate reserves were deficient in relation to carbohydrates for any considerable length of time, yields of fruit were less than when the deficiency had been promptly corrected.

12. Even when the concentration of nitrate in the plants was extremely low and carbohydrates were also deficient, the addition of nitrogen—whether or not it increased the nitrate reserve—did not result in materially increased yields. In some cases there was an apparent decrease in yield.

13. There was some evidence to indicate that soil temperatures of 68° F. or lower result in limitation of absorption of nitrate by the roots.

14. During seasons when minimum temperatures are not low, plants deficient in nitrate and high in carbohydrates may fruit months earlier than others that contain a more liberal nitrate reserve but a lower concentration of carbohydrates. Following a single night of relatively low temperature or several nights when the temperature was not so low, however, plants comparatively high in nitrate—as well as others that are lower—may apparently differentiate flower primordia simultaneously.

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MEIOTIC COILING IN TRADESCANTIA

C. P. SWANSON

(WITH NINETEEN FIGURES)

Introduction

Despite considerable research on chromosome coiling (see **7, 9, 16** for reviews), apparently no strictly quantitative attack on the problem has as yet been attempted, although this method offers one of the better avenues of approach to a more complete understanding of the coiling mechanism. It seemed worth while, therefore, to investigate the effects of a wide range of temperatures on meiotic coiling from a quantitative as well as from a qualitative point of view. This paper deals largely with the data thus obtained, together with data on the origin and development of the major coils in meiotic prophase stages. Some quantitative data on the genotypic control of major coiling are also included.

MATERIAL AND METHODS.—The genus *Tradescantia* has been used exclusively in this study. Two clonal lines were available, one of *T. paludosa* Anders. & Woodson and the other of *T. canaliculata* Raf. The meiotic chromosomes of the latter were statistically longer than those of *T. paludosa* and consequently were more easily analyzed, so that this line was more extensively used than the other. The results from both clonal lines, however, were closely parallel in all respects.

The plants were grown in pots during the fall and winter of 1940–41 in the greenhouses of the Biological Laboratories, Cambridge, Massachusetts. The average temperature of the houses was approximately 22° C. during the day but fluctuated so much during the time of the experiment that data from buds taken directly from the greenhouses were of little critical value. A considerable amount of data was gathered from such buds, however, and the average of these figures was used as an arbitrary control. For temperatures above greenhouse range, heat chambers were utilized, while constant-temperature coldrooms permitted the use of temperatures below 22° C. Both whole plants and cut inflorescences provided the cytological material, and since the results from both sources showed no significant deviations, the data were considered together.

TERMINOLOGY.—During meiotic prophase the number of major coils was greater than at meiotic metaphase. The prophase coiling cycle in meiosis (and mitosis as well) in *Tradescantia* is characterized by a progressive reduction in the number of coils. It is therefore necessary to conceive of the chromosome during the coiling cycle—not as a system becoming progressively coiled or spiralized as it passes from prophase to metaphase—but as one becoming progressively uncoiled or de-

spiralized. It has also been necessary, as a result of these observations, to separate the prophase coiling cycle into two stages: (1) the spiralization phase, at which time a relatively straight chromonema is transformed into a regularly coiled system; and (2) the despiralization phase, during which the numerous coils formed during the spiralization phase are reduced by an uncoiling which must take place at the distal ends of the chromosome arms, and which undoubtedly is accompanied by a rotation of ends within the matrix.

Observations

Genotypic control of major coiling

Numerous instances of an intraspecific genotypic control of meiotic chromosome length have been reported since Lesley and Frost's (**11**) classical case in *Matthiola incana* (**2**). In this plant the long chromosome form was recessive to the normal short chromosome form. A similar instance of altered chromosome length acting as a single Mendelian recessive has been described by Upcott (**24**) in *Lathyrus odoratus*, although the reverse was true in this case, the normal form possessing long chromosomes and the mutant male-sterile for the short chromosomes. Lamm (**10**) found a wide range of meiotic contraction in various segregates of inbred stocks of rye. In *Macronemurus*, a Neuropteran insect, Naville and de Beaumont (**14**) showed the chromosomes in the male to be more contracted than in the female.

In *Tradescantia*, different chromosome lengths were observed in the two clonal lines utilized in this study. Under average greenhouse conditions, *T. canaliculata* showed 8.1 major coils per chromosome (fig. 6), and *T. paludosa* an average of 6.08 (fig. 7), the length of the metaphase chromosomes being correspondingly long or short. Only metaphase chromosomes were used in determining counts, since it was found that the number of coils is progressively reduced during anaphase (fig. 8). The number of coils for *T. canaliculata* is much above the figures given by other investigators for diploid species of *Tradescantia* (**15, 17**).

The two clonal lines were crossed, and F_1 and F_2 populations were grown, although the number of plants in each generation was rather small. Six F_1 plants gave varying counts in the number of major coils at metaphase, the figures ranging from 6.56 to 7.64. In this respect they were intermediate between the two parental averages, but there was a noticeable trend toward the longer chromosome form of *T. canaliculata*. Little significance can be attached to this because of the few plants used and because of possible temperature fluctuations. Eighteen F_2 plants, obtained by crossing the plants with 6.56 and 7.64 coils, respectively, showed an extremely wide range of coiling, the lowest being 4.4 and the highest 7.64 coils per chromosome. Eight out of the eighteen plants showed a lower number of coils than did *T. paludosa*. The data are inadequate for drawing definite conclusions as

to the exact genotypic control, but it is at least apparent that—unlike the instances cited in *Matthiola* and *Lathyrus*—the situation is more complex than a single gene difference, and that both parental plants are heterozygous for genes influencing the degree of spiralization.

From numerous observations on other species of *Tradescantia*, *T. paludosa* appears more nearly to represent the average degree of spiralization for the genus, and apparently the increased length of the *T. canaliculata* chromosomes is due to a difference in the timing relationships during prophase. This difference may be expressed as: (1) normal spindle formation with a slower rate of despiralization, or (2) normal despiralization with a precocious spindle formation. Either would lead to increased chromosome length at metaphase. These observations in no way imply that spindle formation exerts influence on the process of despiralization during prophase. However, since only metaphase configurations were used in this study, since metaphase is a relatively brief yet conspicuous stage in meiosis, and since the onset of metaphase is governed by the development of the spindle, it seems justifiable to view the rate of despiralization and the time of spindle formation as the two most important factors which determine the degree of coiling to be found in any particular cell at metaphase.

To distinguish between these two factors would require a more intimate knowledge of prophase development in *Tradescantia* than is available at present. Upcott (**24**) has been able to ascertain with considerable exactitude the behavior of the normal and the short-chromosome mutant in *Lathyrus*, and she finds that the timing of prophase, as judged from anther size, is such that in the mutant the time period for despiralization is so lengthened as to permit the chromosomes to pass the stage of normal contraction and reach an abnormal contracted state. No detectable difference in anther size could be found between the two species used in this study. When comparing the preceding examples with *Tradescantia*, it is, of course, assumed that the difference in length of the meiotic metaphase chromosomes is directly related to differences in the degree of major coiling, although it is also possible that the chromonema length is under genotypic control (**26**).

Environmental control of meiotic coiling

The ease with which the synchronization of meiotic phenomena may be disturbed is indicative of the delicate balance of the entire mechanism. Of the many environmental agents utilized for experimental purposes (**18**), none—with the possible exception of colchicine—shows any specificity of action, but all express their effect as a general upset in both cell and nuclear division. Recently Giles (**5**) has pointed out that this may be because their ultimate effect is on the hydration mechanism of the cell, which in turn is intimately associated with cytokinesis, spindle formation, and coiling (**20, 9**). Any agent that could alter prophase timing

relationships would show its effect through the coiling system, but in selecting an agent for a quantitative study of the environmental control of coiling, one necessary criterion was that, when varied, it would provide detectable differences of a uniform and continuous nature without totally disrupting the other cell processes. The use of temperature satisfied this requirement. Temperature also has the advantage of being readily controlled, as well as providing a wide and continuous range of conditions within which the plant can function properly.

With the exception of studies on chiasma frequencies (**22, 25**), most cytological investigations of thermal treatment of meiotic cells have been qualitative (**18**), with descriptions of the irregularities observed throughout the various stages of pollen development. Comparatively little has been done quantitatively, perhaps largely because the changes arising from such treatment do not readily lend themselves to quantitative analysis. The coiling mechanism, however, provides a phenomenon which can yield an effective measure of the differences induced under controlled conditions, for as NEBEL (**15**) has already pointed out, and as this study shows, the number of coils per chromosome under constant conditions is a relatively stable and constant character. MATSUURA (**12, 13**), HUSKINS and WILSON (**6**), and WILSON and HUSKINS (**26**), using *Trillium*, were the first to present data showing the effects of thermal treatment on the number of coils. MATSUURA found that treatment at 16° C. (18° C. being considered normal) for 2 weeks reduced the number of major coils to 64 per cent that of normal, whereas 2 months at the same temperature reduced the number to 44 per cent. WILSON and HUSKINS showed that low temperatures lessened the number of coils as well as shortened the length of the chromonemata, while temperatures of 20° C. frequently prevented coiling. This latter effect is similar to that found in *Fritillaria* by BARBER (**1**), in that prolonged treatment (2 days at 30° C.) brought about complete suppression of spiralization in some cells, while abbreviated heat shocks had no visible effect. STRAUB (**22**) found a mitosis-like type of coiling at high temperatures and greater contraction at subnormal temperatures. In root-tip cells of *Allium*, DARLINGTON and LA COUR (**3**) observed that the chromosomes were markedly shortened when subjected to 0° C. for 5 days, although they also stated that at this "marginal temperature" the degree of coiling was variable.

There seems to be some consistency, then, as to the effects of temperature on the visible expression of coiling. Low temperatures reduce the number of major coils as well as shorten both chromosome and chromonema lengths, while high temperatures may completely inhibit the development of coils or cause the meiotic chromosome to assume a mitosis-like form. Since the qualitative effects seem well established, the main problem of the present research was therefore a quantitative one, with observations to be made over a wider and more continuous range of temperatures. The effects of thermal shock were disregarded in this study, and to al-

low for this, the plants were grown for at least 2 weeks at each temperature before any observations were made. Cold-treated plants were allowed longer periods of time to allow for the slowing down of the meiotic process which normally occupies a period of several days, although the exact meiotic timing relationships are uncertain because of the inadequacy of prophase configurations. This would permit examination of cells which had come up from a premeiotic state and had passed through the complete meiotic prophase cycle at the desired temperature.

No attempt has been made to measure chromonema lengths in the manner that HUSKINS and his co-workers have done in *Trillium* (**6, 21, 26**). In fact, in a double-coiled system of major and minor coils, such as is found in *Tradescantia*, measurements of chromonema lengths are virtually impossible since the minor coils are

TABLE 1

Temperature (° C.)	T. canaliculata		T. paludosa	
	No. of chromosomes	Coils per chromosome	No. of chromosomes	Coils per chromosome
8	25	5.92	100	4.02
15	50	7.36	100	5.17
22	150	8.10	125	6.08
24	25	8.52	.....	.....
27	25	8.92	25	6.88
29	18	7.11	10	6.60
33	50	7.64	50	5.96
34	75	6.04	.....	.....
36	75	5.17	75	6.12
40	40	4.15	100	5.10
42	195	4.83	.....	.....

rarely so clearly differentiated that the diameter, pitch, and number of coils can be readily ascertained. The data and conclusions presented here are therefore derived from observations of major coils only, and—as a rule—where the major coils are reduced in number, the chromosome length is similarly reduced. Also, as the major coils are numerically reduced, the diameter of the major gyre is correspondingly increased, and it is probable that the proximal gyres achieve a greater diameter at the expense of the distal ones, which become progressively uncoiled.

The data from both clonal lines of *Tradescantia* are given in table 1 and presented graphically in figure 1. In compiling the data, the number of coils was always interpreted in terms of whole numbers, no attempt being made to determine half coils. In other words, the number of coils in a single chromosome might be 6 or 7 but not 6.5; this leads to some error, but it would be unavoidable in any case, because of the difficulty of following the coils.

From figure 1, it is evident that an essentially unimodal curve holds for both

species when the number of major coils per chromosome is plotted as a function of temperature. The peak in both instances falls at, or around, 27° C. Figure 9 illustrates a typical cell of *T. canaliculata* at 27° C., showing the large number of coils and the slenderness of the chromosomes; figure 10 shows a similar cell at 33° C., the coil number being somewhat below that normal; figure 11 illustrates the reduced length and coil number at 8° C. In this respect the data do not bear out completely previous observations, for here both extremes of temperature lead to greater despiralization and hence to greater chromosome contraction.

No immediate explanation is at hand as to why the peaks of the curves should fall at 27° C. The answer probably lies in the relative effects of different thermal

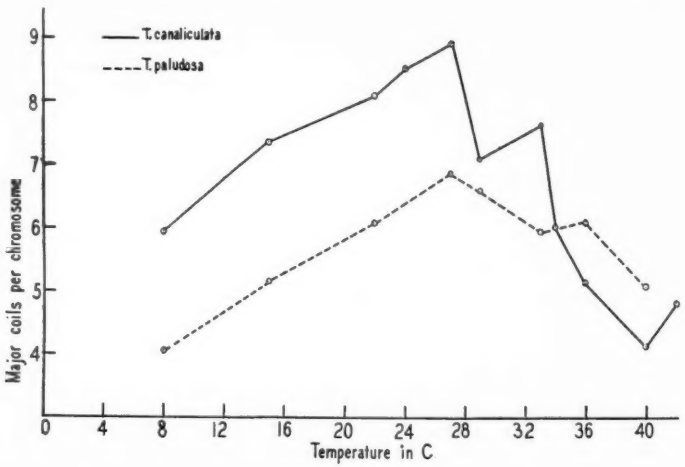


FIG. 1.—Graph illustrating effect of temperature on major coiling

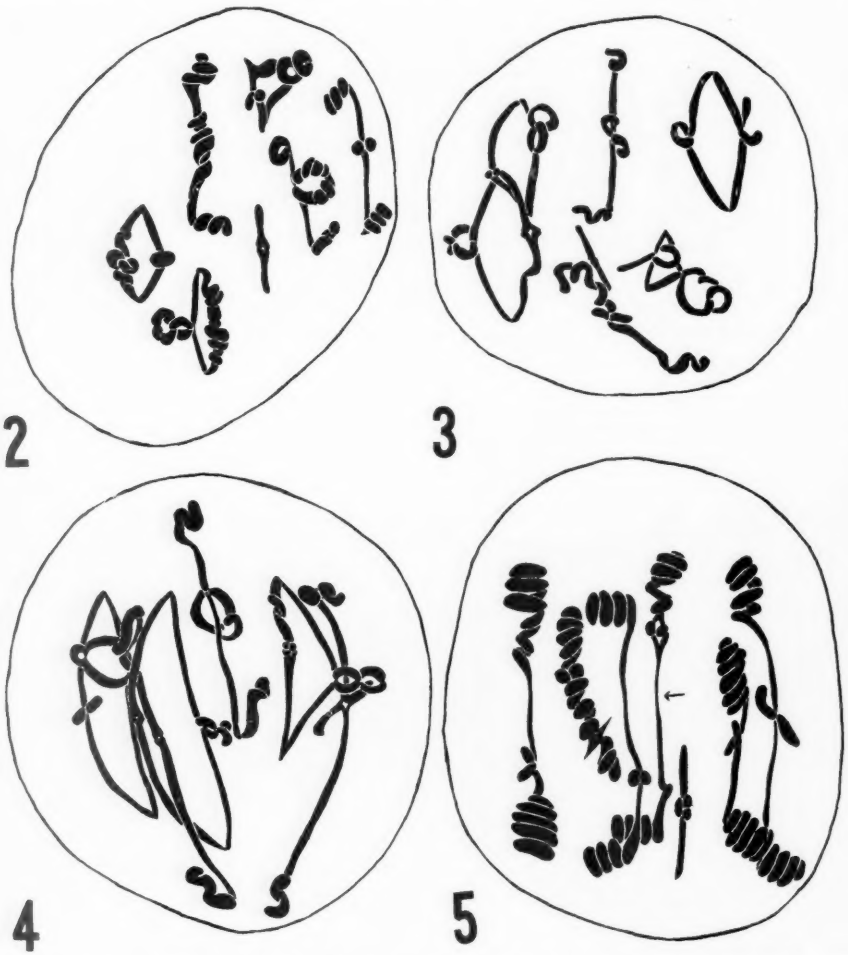
conditions on both the despiralization mechanism and the time of formation of the spindle, but as yet comparatively little is known of the chemical and physical nature of these processes, so that any hypothesis at this time would be suggestive only. In the light of other information, however, it seems likely that low temperatures delay the appearance of the spindle in some manner, thus permitting the coils to go beyond their normal stage of development, and giving in this way a shortened chromosome. At high temperatures, where the effect is more variable (fig. 1), the influence is an accelerating one but is more pronounced on the coiling system than on the spindle, with the result that the rate of despiralization is more rapid relative to spindle formation, thus permitting greater contraction—not because of a greater time interval but because of an increased despiralization tempo.

Variability in the degree of despiralization is greater at high temperatures. This

process must itself be, or is the result of, a chemical process, possibly due to matrical contraction or accumulation of nucleic acid around a protein core; but because all the coiling considered in this study was from metaphase configurations, and hence the expression of two inseparable variables (the coiling mechanism and spindle formation), it was impossible to obtain a temperature coefficient for chromosome contraction. Two slides of *T. canaliculata*, from inflorescences kept at 3.5° C., gave an average of 7.07 coils per chromosome, indicating a possible rise at extremely low temperatures; but since the inflorescences were at this temperature for only a week, and since circumstances prevented a checking of the data, the significance of this figure is questionable, and it was not plotted on the curve. A rise was also indicated in *T. canaliculata* at 42° C., but since temperatures above this killed the meiotic cells, no further data were obtainable. A lower chiasma frequency and a higher univalent frequency were usually found in cells which showed a low number of coils at both temperature extremes. The correlation was so striking as to indicate the probability that much of the movement of chiasmata, and possibly chiasma loss, is directly the result of the coiling mechanism (**23**). This, however, will be discussed at length in a later paper.

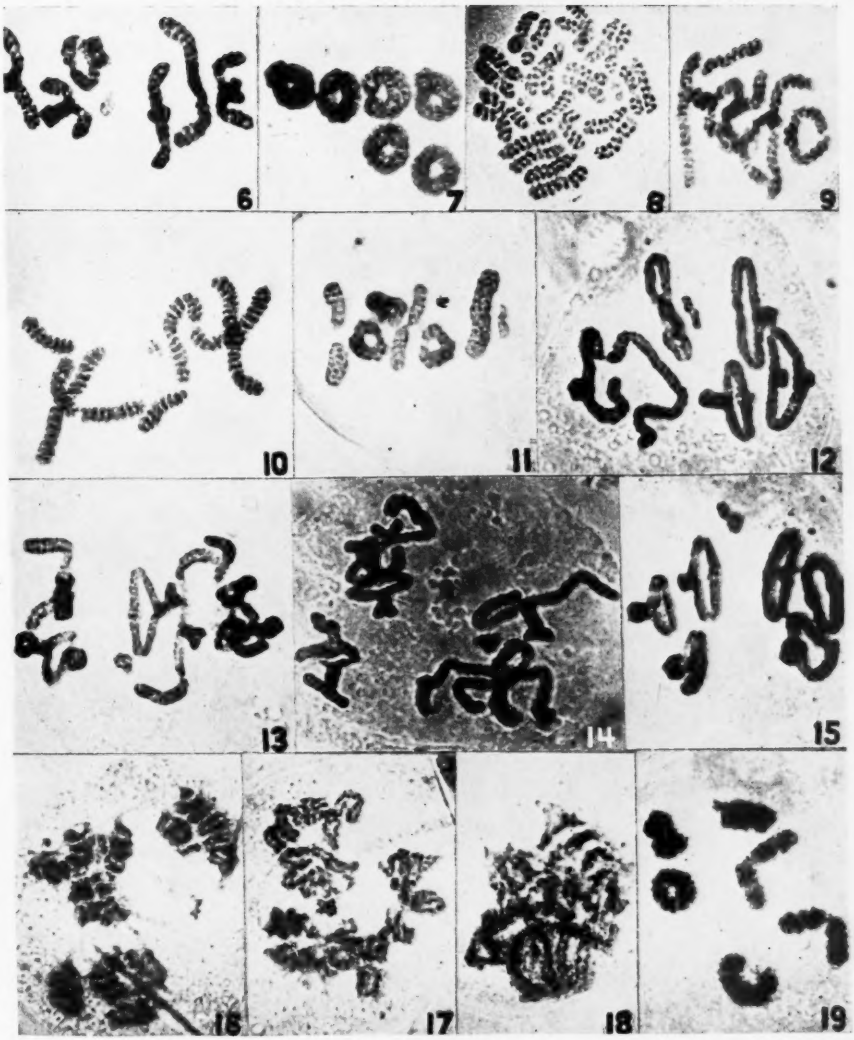
With the exception of univalents, the meiotic divisions throughout most of this thermal range were regular and constant, once the plants had recovered from their transfer from greenhouse temperatures. There was no noticeable upset of nuclear and cytoplasmic synchronization such as is characteristic of thermal shocks (**18**). At temperatures of 40° C. and above, however, striking differences were found in preparations from some buds in inflorescences that had otherwise almost normal material. The figures in table 1 and the points on the curves of figure 1, obtained at 40°–42° C., were included because they were judged to possess a normal, though somewhat increased, state of chromosome despiralization; but other slides at these temperatures were so anomalous as to be excluded from any such consideration. The behavior of these striking cells is to be seen in figures 2–5 and 12–19. All variations in the degree of despiralization were to be found, sometimes in a single anther, the number of major coils varying from thirty-two to as low as two per chromosome. The long chromosomes (figs. 3, 4, 12–15), designated l-chromosomes from here on, appeared mitosis-like, except that they were paired as bivalents, whereas others appeared uncoiled to such an extent that the major coils were almost eliminated. This may be seen in figure 17, where the upper bivalent has the left-hand homologue spiraled into about one and one-half large coils while the right-hand homologue has lost its major coils. This figure also shows clearly the sister half-chromatids, which can separate laterally without entanglement. In some of the more sharply stained half-chromatids there is a suggestion of a third split (long arrows), giving an eight-parted chromosome possessing eight quarter-chromatids; but more observations are needed to make certain of this. The short

chromosomes (s-chromosomes) frequently show the minor coils, as might be expected under such conditions of extreme despiralization (fig. 16). Apparently in



FIGS. 2–5.—*T. canaliculata*, 40° C. Fig. 2, loose and irregular heat-induced coils. Fig. 3, early anaphase; lack of despiralization brought about by heat; lowermost bivalent failed to congress properly. Fig. 4, anaphase; marked lack of despiralization. Fig. 5, anaphase; considerable despiralization.

Tradescantia the minor coils follow a development parallel to that of the major, in that they reduce in number and increase in diameter as prophase progresses. This conclusion is based largely on inference rather than on direct observation, for the



FIGS. 6–19.—Fig. 6, *T. canaliculata*, normal metaphase. Fig. 7, *T. paludosa*, normal metaphase. Fig. 8, *T. canaliculata*, normal anaphase. Fig. 9, same, 27° C. Fig. 10, same, 33° C. Fig. 11, same, 8° C. Figs. 12–14, same, 40° C. Note lack of despiralization at high temperatures. Fig. 15, *T. paludosa*, 40° C. Fig. 16, *T. canaliculata*, 40° C.; arrows point to minor coils; marked despiralization. Fig. 17, same, 40° C.; short arrows point to sister half-chromatids; long arrows point to regions where further division has produced sister quarter-chromatids. Fig. 18, same, 40° C.; early diplotene; incipient major coils appear as dotlike chromomeres on parallel chromatids. Fig. 19, same, 40° C.; diakinesis; marked despiralization. ×1000.

minor coils are difficult to follow in ontogeny; but in cells where the minor coils are greater in gyre diameter and fewer in number than in cells less advanced in prophase, it seems reasonable to assume that the minor coil development parallels that of the major coils, and that the fewer larger minor coils were derived from the more numerous smaller coils by a process of despiralization. This is not in agreement with COLEMAN and HILLARY'S (4) work in *Trillium*, for they state that the minor spiral decreases in diameter to give additional length to the chromonema.

Occasionally, after heat treatment, cells were found in various prophase stages not normally well differentiated in *Tradescantia*. Figure 18 is, so far as can be determined, an early diplotene configuration, with the chromatids visibly separated and the major coils at an incipient stage. They appeared to have been "thrown in," rather regularly, on the previously uncoiled chromonemata. Figure 19 shows the widely distributed chromosomes at diakinesis. This stage appeared frequently at 40° C., and although the bivalents possessed the fuzzy appearance characteristic of diakinesis, some were sufficiently well differentiated so that the minor coils could be detected. Later it was found that the same prophase stages, as well as the coiling anomalies, could be induced by transferring cut inflorescences from the greenhouse to an oven held at 40° C. for 24 hours, as well as by growing the entire plant at 40° C. for 2 weeks.

Even though at 40° C. the coiling system seemed at first to show all degrees of despiralization, analysis indicated that the cells could be separated into two distinct groups with very little overlapping: (1) those cells with greatly contracted chromosomes (considerable despiralization), and (2) those with greatly elongated chromosomes (very little despiralization). Evidently a single bud, or possibly a single anther, contained cells destined to go through division on successive days. Those with the s-chromosomes were to have divided normally on the day that the preparation was made, whereas the cells with the l-chromosomes were to have undergone division the following day; but, owing to a precocious formation of the spindle brought about by the thermal treatment, the chromosomes were advanced a whole day in development, being oriented by the spindle in a partially uncoiled state. It can be stated with some certainty, then, that the effect of the heat in the l-chromosome cells was to accelerate development of the spindle relative to the rate of despiralization, but the exact effect on the s-chromosome cells cannot be so determined. The state of hydration of the chromosomes and spindle probably determines the effect which a high temperature will exert at any particular stage of meiotic development.

Equally striking were the differences in chiasma frequency and distribution between these two cell types (table 2). The l-bivalents probably reveal the approximate position of original chiasma formation, and, as can readily be seen, the chiasmata are largely subterminal. Not infrequently, however, cells are found that show

bivalents with chiasmata formed in the region of the centromere, some of them being extremely close (figs. 3, 13, 14). At normal temperatures never more than two chiasmata per arm have been reported, but these preparations regularly show three in some arms (figs. 3, 4, 13), and rarely four have been found. Seven chiasmata per bivalent has been the highest observed in this material.

Treatment of cells at 40° C. for 24 hours did not markedly increase the total chiasma frequency (table 2), indicating possibly that the l-chromosomes had already undergone chiasma formation at the time of treatment; but the difference in distribution, whether terminal or interstitial, between the s-chromosome and con-

TABLE 2

CHIASMA FREQUENCIES, T. CANALICULATA, AT 40° C.

	Interstitial chiasmata	Terminal chiasmata	Univalents	Total chiasmata
Whole plants grown in greenhouse kept at 22° C. (control)	4.14	7.37	0.33	11.51
Cut inflorescences kept in oven at 40° C. for 1 day				
Slide no. 1 l-chromosome*	8.45	3.21	0.55	11.66
s-chromosome	1.96	7.92	1.20	9.88
Slide no. 2 l-chromosome	9.70	2.90	0.72	12.60
s-chromosome	1.35	8.67	0.80	10.02
Whole plants grown in heat chambers at 40° C. for 2 weeks				
Slide no. 1 l-chromosome	9.50	4.20	0	13.70
s-chromosome	2.08	6.72	1.28	8.70
Slide no. 2 s-chromosome	1.60	7.18	1.33	8.78

* The l- and s-chromosomes are environmental, not genetical, variants and not to be confused with the l-chromosome mutant in *Matthiola* (**11**).

trol cells and the l-chromosome cells strongly suggests the influence of despiralization on the movement of chiasmata. When plants are grown for 2 weeks at 40° C. (table 2), there is a statistically significant difference in total chiasma frequency between the l- and s-chromosome cells, and—as the increase of univalents would indicate—there seems to have been a loss of chiasmata with contraction. The univalents in these cells probably arise from previously paired chromosomes by a loss of chiasmata. A loss of chiasmata by despiralization is also seen by comparing chiasma frequencies of the l- and s-chromosome cells kept at 40° C. for 24 hours. A comparison of the data on l-chromosomes (40° C. for 2 weeks) with that on l-chromosomes (40° C. for 24 hours) and the controls indicates that chiasma frequency is considerably increased when the entire prophase development takes place at higher temperatures (**22, 25**).

The l-chromosomes result, as has been stated, from a precocious spindle formation. Under these conditions the appearance of some abnormalities in congression and orientation of the bivalents would be anticipated, since the time allotted for these activities must necessarily be sharply limited. Observations bear this out. In figure 3, one of the rod bivalents has failed to show congression on to the plate, possibly owing to the long and sinuous length of the arms, which would inhibit movement, as well as to a restricted time period, which would necessarily limit the distance through which the chromosomes could move. The arms show no tension, although the bivalent presumably lies within the spindle. At anaphase the arms become extremely long and slender under the tension exerted by the spindle (fig. 4), and the threads appear about to break, as BARBER (1) has shown in heat-treated material of *Fritillaria*. The chiasmata seem unable to terminalize, the strength of union at the point of chromatid exchange being strong enough to resist the separation force of the spindle. The chromosome itself then breaks at the point of greatest stress, this point depending upon its position on the spindle and the position of the most proximal chiasma. BARBER explains that the anaphase tension begins before the chromatid attraction has an opportunity to lapse.

The s-chromosome cells likewise are not without their abnormalities. In some cells the major coils appear unstable, pulling out under the stresses imposed by the spindle, while free arms remain tightly coiled (figs. 2, 5). The chiasmata fail to terminalize, and the threads between the centromere and the first chiasma become unduly stretched, possibly leading to later breakage, although this has not been seen. Figure 5 illustrates the double function of the spindle in effecting chromosome separation. The second bivalent from the left has failed to congress properly on to the plate, with the result that the centromere of the lower homologue occupies a central position on the plate instead of being equidistant from the plate with its homologue. The unoriented homologue behaves as a univalent, independent of its attached partner, and its centromere divides. This behavior is in agreement with SCHRADER'S (19) observations that the centromere is essentially a double structure at metaphase, and its ultimate behavior must therefore depend entirely on its orientation in relation to the plate and not on the singleness or doubleness of its structure. Such behavior was not observed in the case of ring bivalents, for here the bipolarity of the structure as a whole, owing to its two centromeres, more effectively brings about the correct congression and orientation. The pushing action of the upper part of the spindle may be inferred from the appearance of the third bivalent from the right (fig. 5). The chiasma can be seen at the end of the slender stretched arm. No force is being exerted on the homologue, which lies above the chiasma, as evidenced from its unstretched condition. On the other hand, the lower homologue is nearing a point of breakage (arrow) due to the pushing action of the spindle being exerted entirely on a single arm.

It was not possible to follow these two kinds of cells beyond anaphase, so that their second division behavior is unknown. Fragmentation of chromosomes was frequently noticed in those cells containing l-chromosomes. This parallels the observations in *Matthiola* (**11**), where the *l* mutant, with its long thin chromosomes, showed considerably more fragmentation than did the normal plant. Some underlying mechanism appears to govern chromosome breakage in both instances, reflecting the instability of the l-chromosomes, whether determined genetically (as in *Matthiola*) or environmentally (as in the present instance).

Development of meiotic coils

Little is known of the actual development of meiotic coils from their inception at early stages to their mature proportions at anaphase. The present uncertainty of a universal principle operative in coiling (**9, 16**) demonstrates the futility of translating unknown prophase stages in *Tradescantia* in terms of known material. However, through the use of the heat treatments described here an experimental tool is available by means of which such difficulties can be overcome, permitting the course of spiralization and despiralization to be traced from incipiency, up to and beyond the normal state of development. The effect of thermal shock was to interrupt the process of meiotic despiralization at various intervals in the course of its development by a precocious or delayed formation of the spindle. This provided a graded series of stages, the integration of which clarifies the prophase coiling cycle. A number of distinct early stages, not at metaphase, add to the observations and facilitate the interpretation.

The coiling cycle during meiotic prophase can be conveniently divided into two stages, the spiralization and the despiralization phases. Theoretically the distinction between the two is obvious, for the first stage is clearly the transformation of an uncoiled structure into one that is regularly coiled, while the second stage is one in which the coils are reduced in number by a process of uncoiling. Actually, however, a sharp delimitation of the two processes is not possible, since—paradoxically—the uncoiling is a continuation of the coiling process, not the reverse of it. That is, if—after a straight thread is thrown into a regular coil—the coiling process continues, not by creating more gyres of the same diameter but by enlarging those already formed, the only means by which this can take place is (1) by elongating the chromonemata, in which case the number of gyres will remain constant; or (2) by allowing the proximal gyres to increase in diameter at the expense of the more distal ones which are progressively uncoiled and lost. The reduction in the number of gyres as prophase progresses indicates that the latter mechanism is operative in *Tradescantia*.

The transformation of the pachytene thread into a coiled structure has been visualized to take the following course. In early diplotene an exceedingly minute coil

makes its appearance. The individual gyres appear as chromomere-like dots on the parallel chromatids (fig. 18), but they can be resolved under the microscope into rather regular coils of equal size and distribution (about 1 μ or less apart). They are not so closely packed as the later coils but show a relatively greater distance between adjacent gyres. No suggestion of a zigzag or wavy outline prior to this stage was observed in any cells, but from their appearance these minute coils seem to have been "thrown in" on the threads, their initial size being about 0.5 μ in diameter. Instances were noted where one chromatid showed a change in the direction of the coil not possessed by its sister chromatid, indicating their independence in coiling. The chromatids lie parallel at this early stage (fig. 18), and there is no evidence of chromatid appression (the pressing of the coils of one chromatid into the coils of its sister chromatid so as to give the appearance of a single coiled structure). An attempt was made to determine the number of these small coils at the time of their origin, but the threads could not be followed throughout their entire length.

These minute gyres of early diplotene are destined to become the major coils of metaphase by a process of despiralization. The essential changes taking place during despiralization are three: (1) reduction in chromosome length, (2) reduction in gyre number, and (3) increase in gyre diameter. A fourth change, an alteration in chromonema length (**21, 26**), may occur, but the *Tradescantia* material was not suited for the detection of such a change if present. The physical basis of despiralization can only be conjectured at this time, although, as has been suggested by numerous cytologists, an explanation might be sought in the behavior of the matrix. In any event, the process of despiralization, with its accompanying changes, is very likely further accompanied by a rotation of the ends of the chromosome arms within the matrix. Where sister chromatids are as intimately coiled as in *Tradescantia*, a rotation of chromosome ends, as the coils decrease in number, is a necessity if entanglement of chromatids is to be avoided, and—as figure 8 shows—little or no entanglement of sister chromatids occurs at anaphase.

The major coils in *Tradescantia* arise as new coils in early diplotene. It seems unlikely, as NEBEL (**16**) states, that they are homologous with the relic coils of mitosis. In the first place, the pachytene chromosomes appear—in those organisms suitable for favorable study—as completely uncoiled structures, lacking entirely the irregular relic coils associated with somatic prophase. In the second place, *Tradescantia* somatic chromosomes usually have approximately twenty to twenty-five coils. The meiotic coils, if derived from these somatic coils, should be equal in number, but numerous instances of thirty or more coils in the present meiotic material tend to favor a *de novo* origin for the major coils.

By diakinesis the major coils have undergone considerable despiralization, and the chromosome length has been greatly shortened. At this time the minor coil

makes its first visible appearance, becoming increasingly evident as anaphase approaches. The major coils continue despiralization well into anaphase, for anaphase coil counts, taken from the same slides as metaphase counts, are always less in number. There was no evidence of a minor coil at the time of inception of the major coil (fig. 18), and unless it is assumed that the early diplotene threads are submicroscopically coiled, the major coil must precede the minor in chromosomal development. This is contrary to the data of COLEMAN and HILLARY (**4**), who demonstrate in *Trillium* that the minor coil precedes the major.

The developmental aspects of the minor coil are obscured because of the difficulties of accurate observation, but by a comparison of early and late stages, apparently the same progressive picture obtains as for the major coil; that is, it is one of despiralization following spiralization. Interesting to note, in greatly despiralized chromosomes, is the presence of a subminor coil running perpendicularly to the minor coil. This third minute coil was clearest in those cells in which the major and minor had been relaxed, and although of considerable interest to coiling mechanics, further discussion must await a more detailed study.

The meiotic coiling cycle of *Tradescantia* differs in several ways from the same cycle as seen in *Trillium* (**4, 6, 21, 26**). HUSKINS and co-workers show that in *Trillium* the number of major coils initially produced is determined by the degree of elongation of the chromonema within a matrix (cf. **4**). A comparison of *Trillium* and *Tradescantia* indicates four main coiling differences. The first, relating to the time of development of the major coil as compared with the minor, has already been discussed. The second lies in the great reduction in chromosome length in *Tradescantia* and the lack of chromosome length reduction in *Trillium*, especially during diplotene and diakinesis. The third and fourth differences are concerned with the later development of the major coils. In *Trillium*, once the major coils have been regularly formed, there is no significant change in number of gyres, and there is no significant change in the gyre diameter. The last three differences are interrelated, of course, and if—as has been demonstrated in *Trillium* and assumed for other organisms—the matrix is the morphological unit determining the coiling behavior, it is evident that the matrix in *Trillium* is a more static structure during the time of major coil formation than it is in *Tradescantia*, where the matrix, in order to bound the chromonema, must contract in length and increase in diameter. In *Trillium*, therefore, it appears that the chromonema is the dynamic unit; in *Tradescantia* it is either the matrix, or both matrix and chromonema.

Certain observations point to the type of coiling present in *Tradescantia*, whether of plectonemic or paranemic spirals (**21**). As already seen, the chromatids coil separately, and the appression of one chromatid coil into that of its sister chromatid occurs as prophase advances. The reticulate appearance of diakinetic chromosomes, as opposed to the single spiraled appearance at metaphase, is evi-

dence of incomplete appression, a phenomenon presumably brought about by progressive dehydration of chromosome and matrix (**9**). The chromatids can therefore readily separate laterally at anaphase on dissolution of the chromosome matrix, but this reveals nothing of the true nature of the spiral. With relaxation of both major and minor coils (fig. 17), however, it becomes evident that sister half-chromatids can likewise separate laterally, a condition which apparently can result only from the chromonemata being twisted in paranemic spiral; a plectonemic spiral, upon relaxation, would leave the sister half-chromatids relationally coiled about one another. According to SPARROW, HUSKINS, and WILSON (**21**), a plectonemic spiral of n gyres will give the equivalent of $2n-1$ relational twists if there are no changes in direction. The sister half-chromatids in figure 17 (upper short arrow), however, show only one relational twist, while possessing ten to twelve minor gyres. It has been shown in *Trillium* (**21**) that the minor coil of the first meiotic division becomes the plectonemic spiral of the microspore division. The difference observed in *Tradescantia* may be real, indicating another point of departure from the *Trillium* type of coiling, or it may be merely apparent, the rotation of the chromosome ends undoing the relational twists and obscuring the true nature of the coils. Further work is needed on this point.

Occasional configurations in heat-treated material are not so readily interpreted in terms of the preceding *Tradescantia*-type coiling cycle (fig. 2). The characteristic small coils are present (fig. 13), but chromosome regions not under anaphase tension seem to be thrown into large, loose, and somewhat unstable coils, as though by adopting these coils the chromosome were being relieved of some internal twist. These coils, in their looseness and irregularity, are unlike the ones in the shortened chromosomes in figures 5, 16, 17, and 19. At this time no explanation can be offered as to their nature or origin, other than that they are heat induced.

A comparison of the development of somatic coils in *Tradescantia* with the meiotic coils reveals certain similarities. KOLLER (**8**) states that mitotic coiling in the golden hamster proceeds in much the manner described here for the major coils in *Tradescantia*, but NEBEL (**16**) considers this to be contrary to that found in somatic chromosomes of plants. He states, "The early standard coils appear to be of smaller gyre but not much, if any, more numerous than the final standard gyres." However, KOLLER's observations on animal chromosomes can be readily corroborated in plant chromosomes by a study of the division of the generative nucleus in *Tradescantia* pollen tubes. Here somatic coiling is easily observed, and there is a marked decrease in the number of gyres at metaphase as contrasted with earlier stages (SWANSON, unpublished). The development of somatic coils is similar to that of major coils, and possibly minor coils, in that once the coils are initiated, the remainder of the coiling cycle is occupied by the despiralization process.

Summary

1. The genetical and environmental control of major coiling has been studied in *Tradescantia* from a quantitative point of view. The number of major coils per chromosome was shown to be under complex genetical control.

2. The use of heat treatments revealed that the coiling cycle during meiotic prophase could be broken down into two stages: (1) the initiation of the coils, the spiralization phase; and (2) the loss of coils, the despiralization phase. It was thus possible to show that the major coils of metaphase are derived from numerous coils of small gyre size which are progressively reduced in number by uncoiling at the same time that the gyres are increasing in diameter. Using temperature from 3.5° to 42° C., it was found that at high and low temperatures the despiralization process was accentuated, leading to greater contraction of chromosome length. The greatest number of major coils was found at 27° C. The effect of temperature on the number of major coils was considered both from the standpoint of the despiralization rate and from the time of spindle formation. At 40° C. the coiling was extremely variable, some chromosomes failing to despiralize while others were almost completely uncoiled. Failure of coiling was likewise observed at this temperature.

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ANATOMICAL AND PHYSIOLOGICAL RESPONSES OF SQUASH TO VARIOUS LEVELS OF BORON SUPPLY

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 536

TAYLOR R. ALEXANDER

(WITH FIVE FIGURES)

Introduction

There are available several summaries of the literature pertaining to boron and plant growth (**2, 4, 5, 16, 28**). Studies on the internal boron-deficiency symptoms have shown that different species may have certain chemical and anatomical responses in common, but at the same time some variations have been observed. External boron-deficiency symptoms have been found to vary somewhat in time of appearance and in the sequence in which organs are affected—depending upon the species, environment, severity of the deficiency, and age of the plant (**3, 7, 16**). EATON (**6**) and others have found that different species grown under identical conditions vary strikingly in the amount of boron absorbed and tolerance to boron concentration, to the extent that plants have been grouped into three categories: sensitive, semi-tolerant, and tolerant. PURVIS and HANNA (**19**) place squash in the sensitive group and state that in greenhouse soil squash gave a favorable response to borax applications.

In this investigation, squash was selected as the experimental plant in an endeavor to establish its external and anatomical response pattern and to compare it with species previously reported. It was also desired to investigate the degree to which different organs of the plant responded, with particular emphasis on the first visible symptoms. Preliminary experiments, in which squash was grown in boron-deficient solutions, demonstrated the extreme sensitivity of this plant to the absence of boron. Microchemical examination of the tissues indicated a great difference in catalase activity between treated and control plants. No mention of catalase was found in the literature related to boron nutrition, hence catalase determinations were included in the experiments.

Material and methods

The material used in these experiments was the Chicago Warted Hubbard squash, a variety of *Cucurbita maxima* Duchesne. Uniform seeds were selected and planted in fine sand on a greenhouse bench, where the seedlings were grown until they were about 2 inches above the surface of the sand, the cotyledons fully expanded, and the first foliage leaf showing. This stage was reached in about 7

days. At that time the seedlings were carefully removed from the bench by flooding the sand away with a slow stream of water, and they were then washed in several changes of distilled water. Four hundred uniform seedlings were selected and transplanted to $\frac{1}{2}$-gallon glazed crocks filled with a complete nutrient solution and accommodating five plants per crock. The plants were supported by paraffined sheet-cork in which holes had been punched, the plants being held upright by a light packing of nonabsorbent cotton around the hypocotyls. The crocks were placed on a greenhouse bench and sphagnum moss packed around them. A thin layer of excelsior covering the sphagnum prevented contamination of the nutrient solutions by the sphagnum. This sphagnum-excelsior packing was kept wet during the course of the experiments in an attempt to keep the nutrient solutions as cool as possible. The method reduced the pot temperatures about 10° C. during hot weather.

The solutions contained $Ca(NO_3)_2$, KH_2PO_4, and $MgSO_4$ at molecular concentrations of 0.006, 0.003, and 0.003, respectively. The minor elements used were manganese as $MnCl_2$, zinc as $ZnSO_4$, copper as $CuSO_4$, and iron as ferric citrate at 0.5, 0.05, 0.02, and 1.0 p.p.m., respectively. Boron was supplied to the control solutions at 0.5 p.p.m. as boric acid. Chemicals were of reagent quality. The pH of the solutions was 4.8–5.0, and the osmotic concentration was approximately 0.7.

All plants were grown on the complete solution (boron included) for 4–5 days, when all solutions were renewed. On renewal, half the plants were changed to solutions without added boron. Roots of the plants placed on boron-deficiency treatment and the crocks containing them had been washed previously with distilled water. The remaining plants continued to receive the complete nutrient solution. The crocks were placed on the bench so that boron-deficient plants alternated with controls in all directions. Iron was added to solutions of both treatments at the rate of 1 p.p.m. every other day, and the nutrient solutions were kept at a given level with daily additions of distilled water. The nutrient solutions were changed again after 5 days.

The experiments were designed to extend 10 days after the boron-deficient series were started, with five harvests, each 2 days apart. Three consecutive experiments, designated I, II, and III, were run during the summer of 1940, and environmental data for each 10-day period were obtained from the U.S. Weather Bureau on the campus. The possible sun hours for the 10-day periods were 146.8, 137.8, and 131.7; actual sun hours were 119.6, 66.0, and 85.8; and the gram calories per square centimeter of horizontal surface were 5392.0, 3648.7, and 3729.4, respectively. Hygrograph records showed the humidity conditions to be similar for all experiments. The floor and walls of the greenhouse were kept wet by frequent sprinkling, and a single thickness of cheese-cloth shade was used when necessary

to prevent wilting. Generally the highest solution temperature recorded each day was around 26° C., but extreme ranges from 23° to 33° C. were noted on the hottest days. Solution temperatures were markedly lower at night.

At harvest, twenty plants were taken at random from each of the two treatments and fresh weights determined. For histological material ten samples were selected from the following plant organs: veins and associated tissue near base of blade, and petioles midway between stem and blade of both the first and second foliage leaves; stem tips; stems just above divergence of first leaf; hypocotyls halfway between the peg and cotyledons; and root tips. Plants in the boron-deficient series ceased appreciable growth after the second leaves had partially developed and the second internode of the stem had elongated. Thus the organs sampled represent the entire treated plant and their control counterparts. Tissues were fixed in Sax's modification of Navashin's solution and stained with a variation of Flemming's triple stain.

Using a modified APPLEMAN (1) apparatus, catalase determinations were made on stem tips including the second leaves, first leaves, hypocotyls, and roots. Duplicate 1-gm. samples, representing twenty plants, were taken from organs which had been cut into small pieces and mixed. Each sample was prepared for analysis by grinding in a mortar for 2 minutes with 1 gm. of $CaCO_3$ and approximately 1 ml. of water. The ground sample was washed quantitatively into a shaking bottle, a total of 15 ml. of water being used in grinding and transferring. The equivalent of 10 ml. of 3 per cent H_2O_2 (Merck), standardized against $KMnO_4$ and neutralized to phenolphthalein with N/10 NaOH, was used in each determination. Gas measurements were made immediately after grinding each sample. The shaking bottle was suspended in a waterbath at 25° C., and shaking was started the instant the 10 ml. of H_2O_2 was delivered to the shaking bottle. The time required for the release of either 25 or 50 ml. of gas, depending upon the tissue, was regarded as an index to catalase activity; the time was recorded in seconds from the incidence of shaking.

Results

EXTERNAL SYMPTOMS

In all experiments, boron-deficiency symptoms were identical in aspect and sequence but differed in time of response and extent. Plants in experiment I usually responded 1 day, and in some cases 2 days, earlier than in experiments II and III. Symptoms as they occurred in experiment I are given.

A brittleness in the above-ground portions of the plant, together with a stunted, discolored root system, accentuated in some roots by laterals near the root apex, were symptoms visible on the second day. On the third day general stunting was noticeable, and the second and younger leaves, involving the stem bud, had de-

veloped a yellow spotting and were slightly chlorotic. The second leaves were cupped downward, roughened, and petioles were thickened. Some roots were enlarged near the tips and were of a spongy consistency. By the fourth day spotting

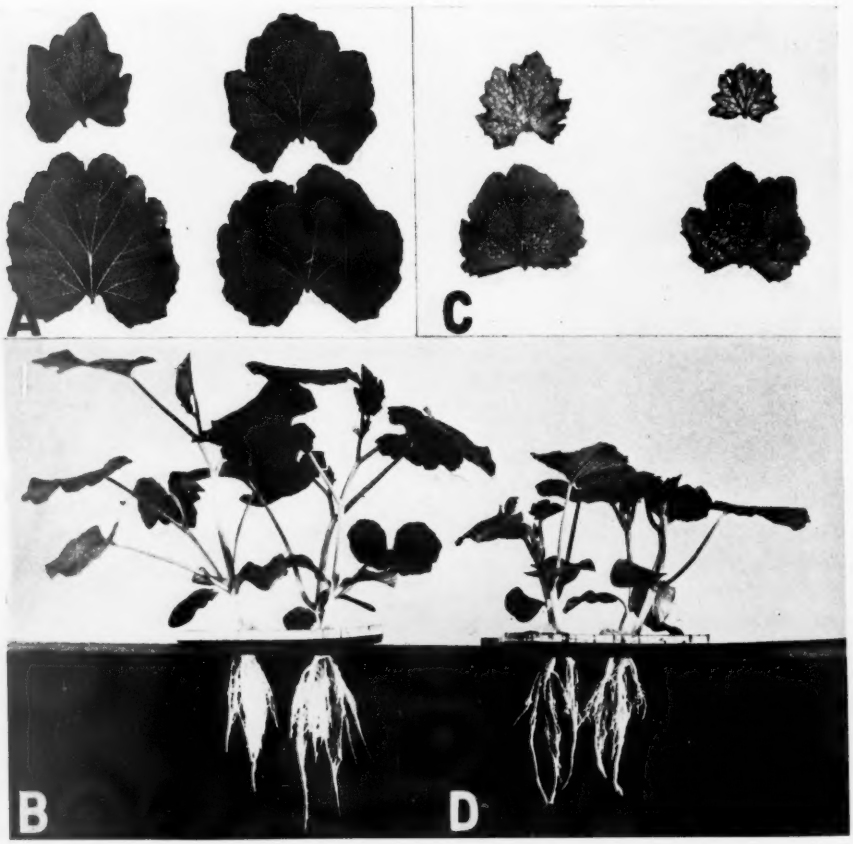

FIG. 1.—External appearance of plants grown 5 days on control and boron-deficient solutions: *A*, second leaves of controls; *B*, control plants; *C*, second leaves of treated plants showing spotting, chlorosis, and stunted size; *D*, boron-deficient plants.

and chlorosis of the young leaves was severe, particularly between the veins toward the base of the blade. Second-leaf petioles and the second internode of the stems were greatly enlarged. A glossy appearance characterized the above-ground portions of the plants (fig. 1). Stem tips had become pale green and soft by the sixth day or were obviously dead.

At the end of the 10-day period all the deficiency symptoms were more pronounced. The control plants had grown until the diameters of their petioles and stems equaled or exceeded those of the boron-deficient plants, which had enlarged rapidly during the first half of the 10-day period. First leaves of the boron-deficient plants remained dark green, stiff, and brittle. The veins of the younger leaves were thickened, contorted, and made prominent by the yellow and chlorotic condition of the vein islets. In all cases, stem tips were dead. Secondary and adventitious roots had succeeded in growing to some extent, but all linear growth had ceased before the experiments terminated. Cotyledons were still green and firm on the boron-deficient plants, whereas they had become wilted and were beginning to fall from the controls.

Plants in the boron-deficient series increased in fresh weight only slighty after the fourth day of treatment, while the controls increased steadily in weight throughout the experiments.

Another difference between the deficient and control plants was their geotropic response. Plants after 2 days in the boron-deficient solutions lagged in their response, while controls responded readily. After 5 days of treatment the boron-deficient plant stems failed to bend, even during a 5-hour exposure to the stimulus.

Internal symptoms

Internal symptoms described in the following paragraphs were observed in material from experiments I and III, with occasional reference to experiment II. Similar symptoms were found in all experiments, the greatest variation being that plants in experiment I gave evidence of response 1–2 days earlier than plants in experiment II or III. All organs which responded extensively to the deficiency conditions were characterized by progressive thickening of cell walls during the first half of the treatment period, separation of liquefied xylem cells by the enlargement of the xylem parenchyma, and the presence of a red-staining substance of granular appearance in the vessels and tracheids. After the plants had been growing 2 days in the boron-deficient solutions, lignified cell walls of the xylem had a greater tendency to retain violet stain than had comparable tissue of control plants. At a given level in an organ, there were instances in which the same tissue varied in its response from no visible symptoms to extreme symptoms. And in general, boron-deficient tissues tended to mature before similar tissues of control organs.

Stem tips.—By the second day, small groups of cells located in the youngest portion of the stem tips were responding in plants of the boron-deficient series, as indicated by their staining qualities; later in the experiment large groups of cells disintegrated in this region. Prior to the fourth-day harvest, unexpanded leaves and leaf primordia had begun to die. In those cases where the primordia

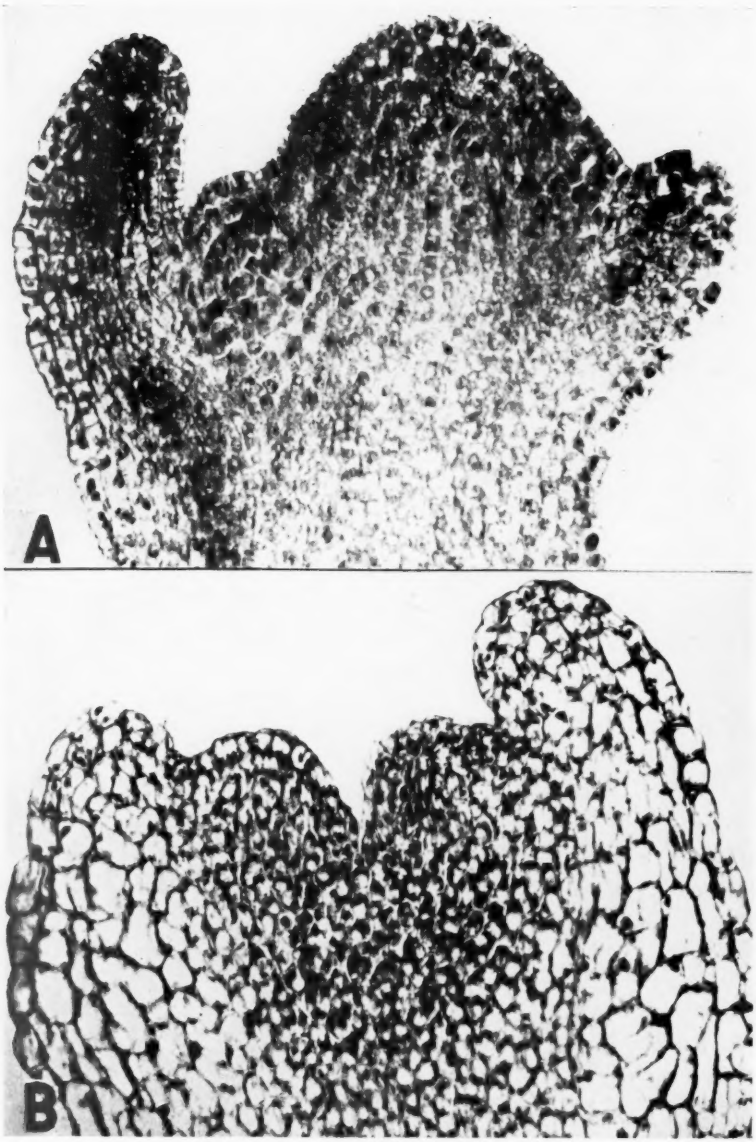

FIG. 2.—Longisections of meristematic regions of stem tip: *A*, control with active embryonic cells; *B*, tip from plant grown 4 days in boron-deficient solutions; cells with disintegrating contents, thickened walls, and frequent cell enlargement; provascular strands not differentiated near apex.

persisted, cells were enlarged, thick walled, and without normal cellular contents (fig. 2). Collapse of the cortical parenchyma, several cells in thickness, had occurred from near the apex to the level of mature protoxylem elements, the dead tissue forming an almost continuous ring just inside the area where a band of collenchyma later differentiates in stems having no boron deficiency. Other cortical

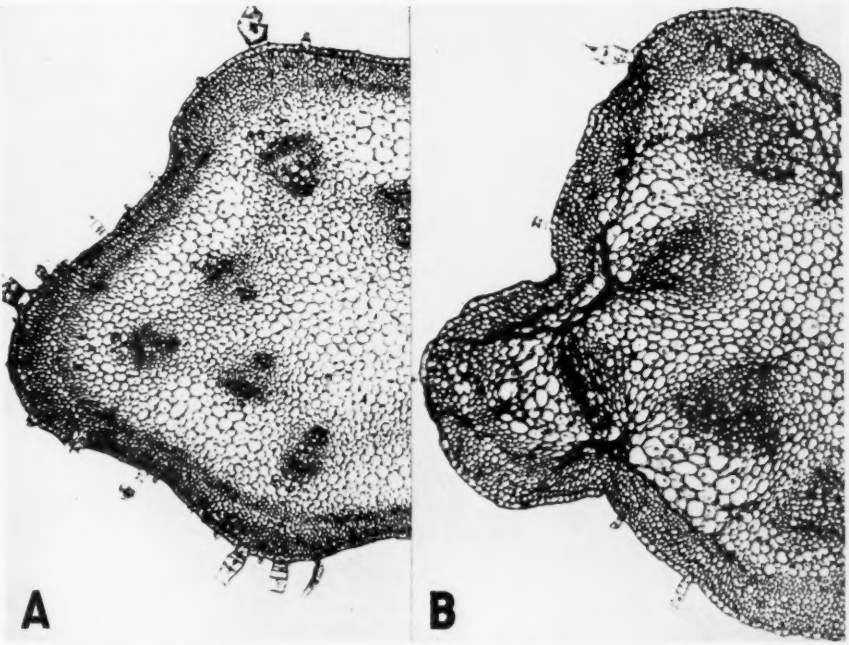

FIG. 3.—Transections from region of maturation in stem tip: *A*, control; *B*, stem from plant grown 4 days in boron-deficient solutions, showing collapse of cells in vascular strands and cortex and enlargement of cortical parenchyma.

cells had thickened walls and were enlarged, whereas cells in the vascular strands and associated parenchyma were dying (fig. 3).

Samples taken on the sixth day showed complete disintegration of large portions of the meristem in the region of greatest cell division. The collapse of the cortical parenchyma, cell enlargement, and disintegration of the embryonic vascular system were more advanced than in former harvests, and in severe cases pith cells were affected. The only additional changes observed on the eighth and tenth days involved death of the tissues at levels progressively farther from the stem apex.

SECOND LEAVES.—Petioles of the boron-deficient plants were larger in diameter than the controls 2 days after treatment was begun. This was attributed mainly to increase in size of fundamental and xylem parenchyma. Collapse of some cortical parenchyma was observed. Plants grown 4 days on boron-deficient solutions possessed petioles which had been further increased in thickness by general hypertrophy of cells of all tissues, especially in the external cambium and the parenchyma between the xylem and the internal phloem. This growth of cells in the vascular bundles had contributed to larger radial and lateral dimensions of the bundles and displacement of the surrounding radially elongated parenchyma, so that in transverse section most bundles were flanked by a group of curved fundamental parenchyma cells. These curved parenchyma cells formed arcs, the centers of which were the vascular bundle (fig. 4). A few tangential divisions had taken place in the lengthened parenchyma cells. Varying amounts of phloem, xylem, and cambial tissue were crushed.

The controls and treated plants had petioles of approximately equal diameter at the end of the experiment. Cell division in the cambiums of the treated plants terminated about the fourth day, whereas the cambiums of the control petioles continued usual development. Increases in size of boron-deficient petioles occurring after the fourth day may be related to the great degree of hypertrophy in the affected tissues.

Marked symptoms had developed in the veins between the second and fourth days. The parenchyma between the adaxial phloem and xylem and the abaxial cambium had elongated in radial dimension, elongation taking place in the order named. This gave rise to a vertically lengthened bundle in which the protoxylem and the most recently formed xylem cells were frequently crushed. Xylem parenchyma cells also became enlarged, as well as the fundamental parenchyma cells adjacent to the bundles. There had been little change in the mesophyll of the boron-deficient leaves since the second harvest. Intercellular spaces were absent, giving the leaf a compact appearance in transverse section. Chloroplasts were small and differed in staining reaction from chloroplasts of control plants.

Progressive changes in the initial symptoms were noted on the sixth, eighth, and tenth days. Greatest increase in cell size had occurred in the abaxial cambium, the cells being radially and laterally enlarged and lacking uniformity of arrangement. The mesophyll cells had grown in size so that they were as large as or larger than the mesophyll cells of control leaves, but there were no intercellular spaces. In contrast, the control leaves were characterized by extensive intercellular spaces.

STEMS.—The stems of the 2-day boron-deficient series were larger in diameter than the control stems and were found to contain enlarged fundamental parenchyma, xylem parenchyma, and parenchyma between the xylem and internal

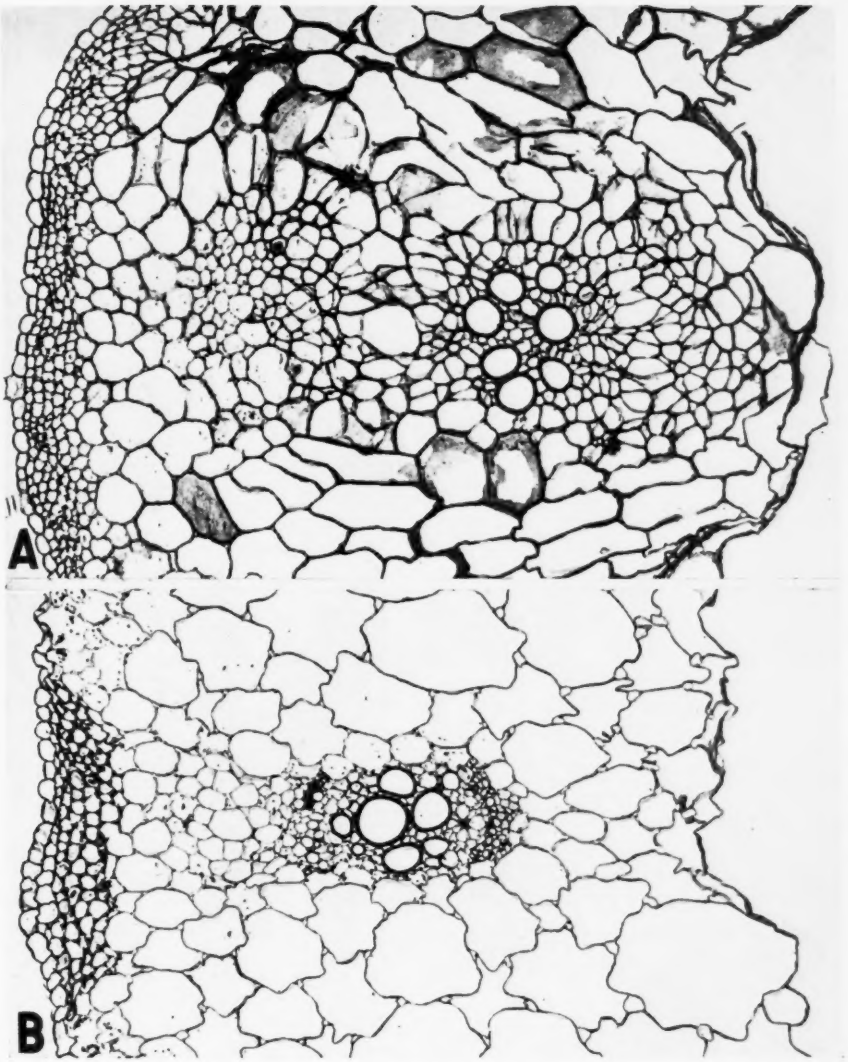

FIG. 4.—Transection of vascular bundle in second leaf petiole: *A*, bundle from plant after 4 days in boron-deficient solutions; note thickened walls, radially elongated fundamental parenchyma, cambium, and parenchyma cells between xylem and internal phloem, enlarged xylem parenchyma, and crushed cells of cortex: *B*, control.

phloem. Two days later the differences were accentuated by continued cell enlargement of these tissues and the external cambium. External phloem cells and cortical cells immediately outside the bundles in which symptoms were evident were compressed radially. On the tenth day the control stems were of greater diameter than the treated, but on any radius the latter were approximately as

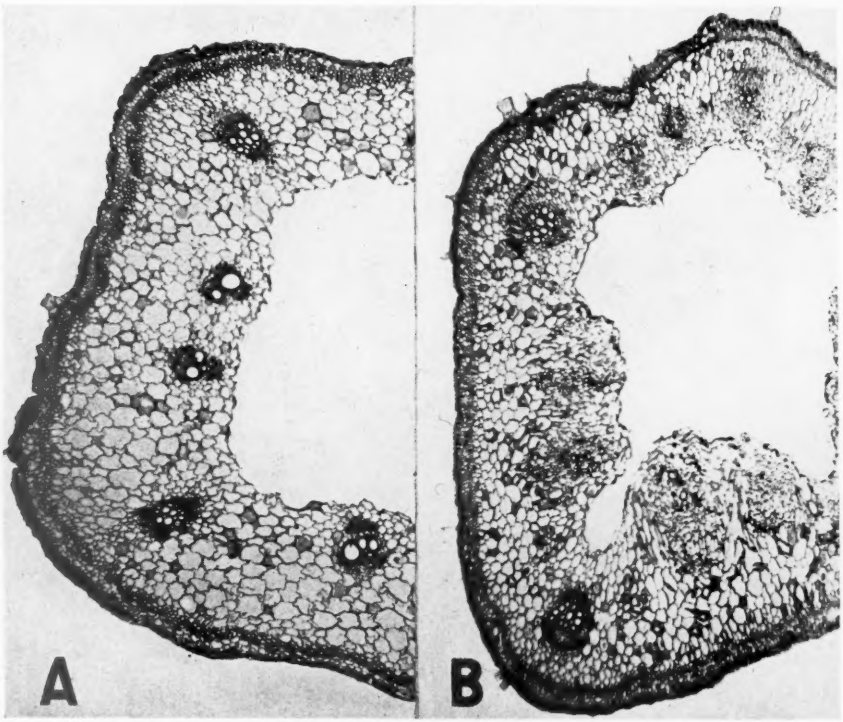

FIG. 5.—Transection of second internode of stem: *A*, control; *B*, stem from plant grown 10 days on boron-deficient solutions; note collapse of cortical parenchyma near upper portion of section and growth of tissue into cavity of stem.

thick from the outer edge of the stem cavity to the outside of the stem. However, unequal centripetal growth of the tissues of treated stems was such that cells in localized areas protruded into the stem cavity, destroying the regularity in thickness observed in the control stems (fig. 5). The bundles which had been largely undifferentiated at the beginning of the treatment consistently had fewer lignified xylem cells than other bundles. These bundles also often contained a group of radially elongated cells located in the region where the external cambium

usually originates. In cortical cells adjacent to or near the ring of sclerenchyma, a collapse of cells which sometimes extended through the collenchyma to the epidermis frequently occurred. These breaks generally described an irregular arc many cells in length (fig. 5).

FIRST LEAVES.—Symptoms of the petioles noted on the last day of harvest paralleled those of the second-leaf petioles. Symptoms made their appearance approximately 2 days later than the latter, however, and were never as great in

TABLE 1

CATALASE ACTIVITY (EXPRESSED IN SECONDS) REQUIRED TO LIBERATE 50 ML. O_2 FOR STEM TIPS AND FIRST LEAVES AND 25 ML. O_2 FOR HYPOCOTYLS AND ROOTS

ORGAN	SECOND DAY		FOURTH DAY		SIXTH DAY		EIGHTH DAY		TENTH DAY		SIGNIFICANT DIFFERENCES	
	+B	−B	+B	−B	+B	−B	+B	−B	+B	−B	1%	5%
	EXPERIMENT II											
Stem tips	10.3	8.5	15.6	4.8	20.1	6.7	15.1	6.9	.....	.....	1.9	1.3
First leaf	26.6	18.5	30.3	26.0	51.0	27.6	45.3	28.5	.....	.....	3.2	2.2
Hypocotyl	94.0	136.0	66.0	50.5	102.1	81.8	139.0	39.7	.....	.....	32.3	22.2
Roots	33.3	58.4	25.0	33.0	37.0	40.9	47.6	41.5	.....	.....	14.9	10.3
	EXPERIMENT III											
Stem tips	10.3	6.3	20.5	8.9	23.9	6.1	13.6	4.5	11.1	8.7	7.0	4.9
First leaf	7.5	6.5	15.3	8.7	15.5	6.5	9.4	5.7	11.7	3.9	1.8	1.3
Hypocotyl	64.0	56.7	91.5	73.0	132.0	48.0	75.0	24.0	54.0	10.0	25.7	18.1
Roots	92.0	81.3	101.0	85.0	88.0	43.0	91.0	56.5	81.0	44.0	10.5	7.4

extent. The mesophyll of leaves from the two treatments was similar in all visible aspects.

HYPOCOTYLS.—As a rule, material from all three experiments failed to show significant differences between the treated and control plants. But an occasional bundle was observed in which there was a suggestion of initial boron deficiency, as had occurred earlier in stem bundles.

ROOT TIPS.—Description of boron-deficiency symptoms in roots is difficult since varying degrees of response could be observed in material of a given harvest. Incipient and advanced symptoms were evident on the second day of treatment. Contrasted with control roots, the histogen region of boron-deficient roots had decreased in extent, and maturation processes were active nearer the apex. At levels where there had been no differentiation of stelar tissues, collapsed, disinte-

grated, and enlarged cells or groups of cells were observed in the peripheral portions of the stele. Hypertrophy of the embryonic stelar tissue and stretching of the slightly enlarged cortical cells by growth of stimulated lateral root primordia which were prominent almost to the tip of some roots, were factors in the increase of root diameter.

Roots examined after 4 days in the boron-deficient solutions were affected severely. Death and disintegration of the extremities of the tip were not uncommon. In the region of elongation and maturation, individual cells were enlarged, others were crushed, and in some roots the central portion of the stele was so completely disintegrated that cells could no longer be recognized. Farther from the apex there was crushing and collapse of cells associated with growth of laterals, and frequently the lateral roots developed deficiency symptoms before emerging from the cortex. These symptoms were characteristic of those found on the remainder of the harvest days, except that deterioration became more extensive.

Chemical measurements

The data on catalase determinations may be found in table 1, where the figures for catalase activity are averages of duplicate samples. The "significant differences" included in the table refer to differences necessary for significance between the control organs and the treated ones on any harvest date. In the cases of stem tips and first leaves, the evolution of oxygen from preparations of boron-deficient tissue was so rapid that it was found necessary to use 50-ml. volumes. Hypocotyl and root tissues were relatively inactive; therefore the volume was reduced to 25 ml. when these tissues were analyzed.

Discussion

Differences in time required for the appearance of both external and internal symptoms of deficiency in experiment I as contrasted with experiments II and III have been mentioned. Warington (**26**) reported that the delay in appearance of symptoms in other seasons compared with summer is correlated with decreased day length. Eaton (**7**) also mentions variations in response between seasons and in growth periods within seasons. Experiment I was conducted under comparatively high light and temperature conditions, and this probably accounts for the discrepancies noted between experiments. The effect of external conditions on catalase activity was not ascertained, since such activity was determined only for experiments II and III, which were conducted during periods of comparable conditions.

In squash the response to boron-deficient nutrition was found to be general throughout most of the plant. All organs responding extensively by the second day had developed entirely or in part after the treatment was started, and the relative maturity of the hypocotyls and first leaves at the beginning of the treat-

ment might have been a factor in limiting their response. Stem and root tips, second leaves, and stems gave anatomical evidence of response within 2 days. Of these, the apical meristems were the most reactive. Disintegration and collapse of parenchyma cells in the apex of the meristem and cortex of the older portions were the first responses in the stem tips; while hypertrophy of xylem parenchyma, fundamental parenchyma, and cambium characterized the earliest symptoms in leaves and stems. The downward cupping of the second leaves after the plants had been growing in boron-deficient solutions 3 days can be correlated with the first visible internal symptom; namely, the increased size of parenchyma cells between the xylem and the internal phloem. In petioles and stems, likewise, comparable tissue tended to be the first to increase in size. The paucity of intercellular spaces in the mesophyll of second leaves under the treatment was probably associated with the failure of these leaves to expand fully.

Hypertrophy and disintegration of the cambium followed by disintegration of the phloem has been reported as an early symptom in stems of *Vicia faba* (**25**), citrus (**9**), and sugar beet (**21**). The short duration of the experiments in this investigation might account for the relatively limited response of the phloem. Ground parenchyma is also frequently affected, as shown by sugar beet (**2, 21**) and *V. faba* (**25**). DENNIS (**4**) quotes work of JAMALAINEN, in which the first symptom of deficiency in Swedish turnip was enlargement of the xylem parenchyma. The collapse of cortical parenchyma in the cortex of the stem and petiole of squash may be similar to the conditions preceding "cracked stem" of celery caused by boron deficiency (**20**).

In the root tip the stele was the region which responded most to the deficiency. Hypertrophy of the cells and stimulated lateral root primordia were the main symptoms. These results are in general agreement with WARINGTON'S report on *V. faba* (**25**) but differ in some aspects from work reported by SOMMER and SOROKIN (**23**). The latter, working with *Pisum sativum*, found the outstanding symptoms to be hyperplasia of the plerome, hypertrophy of the periblem, and abundant primordia of secondary roots near the apex. ROWE (**21**) stated that sugar-beet roots merely failed to elongate in boron-deficient solutions. The fact that varying degrees of response in root tips were observed during the early phases of the present experiments has been mentioned previously in the account of internal symptoms. Some of these differences may be related to variation in response between individual plants. It was noticed after treatments were started that the boron-deficient plants frequently developed adventitious roots from the hypocotyl. These roots grew rapidly for a short time, while other roots had ceased terminal growth. The adventitious roots were occasionally sampled with the other roots, and this might also have been partially responsible for some variations seen in anatomical symptoms between and within harvests.

With the exception of the first harvest date, significant differences in catalase activity of the stem tips and first leaves were obtained between the treated and control plants. The data (table 1), however, show that on the first harvest, differences were either significant or approaching significance at 5 per cent. Samples prepared from the treated tissues were always more active than those from control tissue in the liberation of gas, the greatest difference occurring about the sixth day. At this time the treated tissues required only about one-third the time that control tissue did to liberate equal quantities of oxygen. Tissues containing the greatest number of chloroplasts were most active. Besides liberating gas slowly, hypocotyls and roots usually gave less consistent results between experiments and harvest dates within experiments. On the other hand, the activity of these boron-deficient organs consistently exceeded the controls on each harvest day of experiment III. The use of entire roots—rather than the root tips—might account in part for their comparative lack of activity. With the exception of root tips, there was a positive correlation between the organs with the first and most extensive anatomical response and those with the highest rate of catalase activity.

The function of catalase in the plant cell has not been demonstrated, although there are numerous reports on the relation of catalase activity to respiration and general metabolism. HEINICKE (**14**) contended that catalase activity is a measure of the metabolic status of tissues and that it might serve as an indicator of the physiological responses of plants to their environment. Considering the occurrence of abnormal growth and disintegration of cells in tissues studied, the following reports are more specific and possibly of correlative nature. HARVEY (**13**) found that "overgrowths" produced by freezing or by *Bacillus tumefaciens* on *Ricinus*, beet, and *Bryophyllum* brought about a lower pH of tissue and increased the activity of catalase over normal tissue. Later it was shown that potato tissue affected with the potato wart disease, which also caused "overgrowths," had twice the catalase activity of normal plant tissue (**27**). A physiological disorder of apples in storage known as "soggy breakdown" is preceded by and associated with high catalase activity, as contrasted with normal apples (**11, 18**). With reference to enzymatic activity and boron nutrition, only one report was found. HAAS and KLOTZ (**10**) reported that citrus leaves from boron-deficient plants exhibited a slightly greater diastatic activity than normal leaves.

The catalase molecule has received considerable attention in recent years. Catalase is now thought to be a chromoprotein composed of a colored prosthetic group, apparently a hematin compound, with a protein (**8**). Hematin is an iron-porphyrin compound (**12**), and the family of iron-porphyrin proteins includes others of the most active catalysts known, for example, peroxidase and cytochrome. There is general agreement that the iron present in catalase is in the ferric state, and the number of iron atoms per molecule is considered to be four

(8). ZEILE and HELLSTRÖM (30) demonstrated positive correlation between enzymatic activity and content of porphyrin-bound iron in catalase from horse liver. Other workers (24) have found that those preparations of horse and beef liver catalase which are the most active produce the greatest quantity of hemin iron.

ZEILE (29) investigated catalase prepared from cucurbit seedlings (Samen der Kurbissorte "Lang gul Märg") and found that it gave absorption bands in common with catalase from horse liver; he concluded that plant and animal catalase are similar. This work was confirmed by KEILIN and HARTREE (15), who prepared their catalase from germinating cucumber seeds.

Examination of the symptoms preceding and coexistent with the increased catalase activity in the boron-deficient plants and the chemical structure of catalase suggested some possible correlations between the extremely high catalase activity and the chlorotic condition of the most active tissues. Chlorophyll has a chemical structure related to the porphyrins, and the present concept of the chlorophyll molecule has such a nucleus (12). Hence, the destruction of chlorophyll or interference with its normal synthesis could lead to the accumulation of compounds related to the prosthetic group of catalase. NEISH (17), reporting on three species of plants, stated that most of the catalase in leaf cells is found in chloroplasts together with a concentration of iron and copper—all of which are oxidative catalysts. The leaves which developed under the boron-deficient treatment failed to develop normal chloroplasts. Further, the necessity of iron for the formation and maintenance of chlorophyll has been established (16), and SHIVE (22) states that functional iron is in the reduced state. If it is assumed that the increased catalase activity represents a larger percentage of total iron in the ferric state than in less active tissues, the inactive condition of the ferric iron might account for some of the chlorosis observed in the boron-deficient squash.

The brittleness of the boron-deficient tissue suggested another possible explanation for the increased catalase activity. It was thought that the brittle tissue might grind more readily and bring about a greater extraction of the enzyme. However, variation in the time of grinding brought about no significant differences in results. The influence of the affected chloroplasts as a factor was not investigated.

Summary

1. Plants were grown 10 days on control and boron-deficient solutions. Samples representing the entire plant were taken every second day for anatomical examination and catalase determinations.

2. External symptoms of boron deficiency were described for the entire plant and were correlated where possible with internal symptoms.

3. Anatomical responses were evident in stem and root tips, second leaves,

and stems 2 days after the boron-deficient treatment was started. In the stem tips, cell enlargement and collapse occurred in the region of cell division and in the cortex of more mature portions. The stelar cells of the root tip were similarly affected. Tissues which were actively growing at the beginning of the treatment were the most responsive. Hypocotyls were very inactive.

4. Hypertrophy of the xylem parenchyma and the parenchyma in the regions where the internal and external cambiums usually originate was the first visible response in organs with differentiated vascular bundles.

5. Boron-deficient organs showed more catalase activity than control organs, the differences being obvious even on the second day of treatment. With the exception of root tips, the greatest catalase activity was measured in the organs with the earliest and most extensive histological responses. These organs were also the ones which became chlorotic, and a possible correlation between this condition and the extreme catalase activity was suggested.

6. Plants of the boron-deficient series soon lost their capacity for geotropic response. The first evidence of this was noted on the second day of treatment.

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ROOT DISTRIBUTION AND ENVIRONMENT IN A MAPLE-OAK FOREST

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 537

NORBERT J. SCULLY

(WITH TWENTY-ONE FIGURES)

Introduction

Various studies have emphasized the importance of investigating root distribution (*a*) as a phase of the structure of communities; (*b*) in correlation with horizonal characteristics of soil profiles which might directly or indirectly affect root growth; (*c*) as a background for the logical sampling of soil factors; (*d*) in interpreting plant competition studies and individual plant responses. All these outlooks are necessary in a complete analysis of environment and community relationships. The present study was approached primarily from the first two points of view but also furnishes a background for further work along other lines.

The trench method of investigating roots (**18**) offers no difficulty when most roots present belong to one or a few dominant species. In deciduous forests with luxuriant ground cover, however, difficulties in root identification tend to limit the value of such study to simple correlation based only on the total root distribution, although many of these difficulties can be overcome by certain modifications and additions to the method.

Investigation of root distribution by the trench method is essentially statistical. Any method employed should measure accurately the variability in the population to be sampled. A refinement of methods to cope with the degree of heterogeneity is desirable. The present investigation offers a partial solution to the problem in deciduous forests, where roots are numerous. Further, it seems desirable that any study of relationship between root distribution and environment should consider the whole biology of the soil profile. Such study demands analysis of the soil proper; of inherent growth tendencies of the root systems of given dominant species as well as their responses to specific soil characters; and—of equal importance in most habitats—the role played by soil invertebrates and burrowing animals. Animal activities are in need of much more study, particularly in regions exhibiting the mull type of forest soil profile.

The present study consists primarily of a root distribution and environment investigation in a 65-acre tract of sugar maple–red oak–white oak forest. Counts were made of all roots occurring in profiles of sixteen selected stations, employing an old and a new method of trench sampling; and an attempt was made to cor-

relate their occurrence in number, size, and area to the respective horizonal features. A number of physical soil factors and the occurrence and role of earthworms and cicada nymphs in the profiles were observed.

The investigation was conducted during the summers of 1938, 1939, and 1940 at Wychwood, Lake Geneva, Walworth County, Wisconsin.

Regional habitat conditions

Climate

Nearly all of Walworth County is included within the Rock River basin. This climatic province has the longest growing season of any in Wisconsin, averaging approximately 170 days (**20**). The mean annual temperature at the Weather Bureau Station at Delevan, a few miles from Wychwood, is 46.0° F. The average annual precipitation of 31.4 inches is distributed rather uniformly throughout the growing season, with an average rainfall of more than 2.6 inches per month from April to September, inclusive. The data from the Delevan station indicate comparatively slight climatic fluctuation.

Physiography

Wychwood lies on the Darien moraine of the "kettle-range," one of the most striking topographic features of a large portion of eastern Wisconsin (**17**). About Lake Geneva, on the north shore of which Wychwood is located, the topography is typically irregular. Within the 65 acres of forest a definite knob-and-kettle topography is found, although there are only a few true kettles. Slopes of 15°–20°, occasionally extending 100 feet or more, are comparatively common. The land rises to the north in the $\frac{2}{3}$-mile length to a maximum of 1029 feet above sea level, or 168 feet above the lake, affording good conditions for drainage. A few depressions without surface outlets occur, but they normally possess comparatively good internal drainage.

Soil

All profiles examined at Wychwood correspond to the Bellefontaine silt loam type (**8, 11, 17**), a characteristic type on the more rolling till deposits in eastern Wisconsin, belonging to the Gray-Brown Podsolic Group. The underlying rock formation, from which the glacial deposits at Wychwood are derived in large part, is Niagara limestone. Owing to the calcareous nature of the parent soil material and the annual return of large supplies of bases to the surface through the decomposition of fallen leaves, a "coarse mull" type (**7**) of soil has developed in which the organic matter is intimately mixed with the upper few inches of the mineral soil.

The A_0 horizon is variable in thickness because of the irregular local topography

and because the annual leaf fall completely disappears, except for occasional petioles, before the next year's foliage is shed. The rapidity with which leaves decompose in this forest during the summer months is unusual. The possible role of earthworms (*Lumbricus* spp.) in this regard is treated later.

The profiles examined typically had a dark gray, silt loam A_1 horizon with an average depth of 4 inches. With few exceptions, the horizon is relatively high in organic matter in the specific stations and in other sampled areas. Numerous small but visible pores were evident, and the soil was distinctly crumbly and friable.

The A_2 horizon, occasionally absent, was a grayish yellow silt loam, indication that leaching had not been complete. It was somewhat lighter in texture than the A_1. Encountered at an average depth of 4 inches, it had an average thickness of 5 inches. Typically this horizon was the lightest colored in the profile and showed a slight blocky structure at its lower levels.

The B_1 horizon, a heavy silt loam, occasionally with varying amounts of sand, varied in color from dull brown to yellowish brown. Encountered at 9 inches, it was 4 inches thick. The soil had a well-marked, small blocky structure which was more evident than in the A_2.

The B_2 horizon, encountered at 13 inches, was 19 inches thick and consisted of a heavy, reddish brown sandy clay. It had a characteristic angular nut structure which became more massive as the C horizon was approached. At the upper limits of the C_1, the nut structure disappeared. Upon wetting, the B_2 horizon becomes very sticky and plastic. Upon drying, the individual blocks shrink and the sutures give rise to marked hair-line crevices. Isolated white to light yellow particles of cherty material of variable size characterized the horizon, particularly in the lower portions.

Appearing at a depth of 32 inches, the C horizon consisted of a gravelly sandy clay or a gravelly sandy loam. Limestone rocks were found in variable numbers and sizes in the upper 6-inch level of the horizon.

This soil type is subject to erosion, evidenced by exposure of the A_2 and, occasionally, B_1 horizons in scattered areas. Erosion is not confined to areas with marked slopes but also occurs on comparatively level surfaces.

Vegetation

Deciduous forest is the climatic climax in the region. In the southeastern portion of Walworth County it was originally represented by a mixture of several species of oak, maple, hickory, etc. (**17**). At present the forest at Wychwood, one of the few relatively undisturbed stands in the region, is primarily sugar maple–red oak–white oak. The older oaks are more than 200 years old, while many maples are 100 years or more.

TABLE 1

RELATIVE ABUNDANCE* AND FREQUENCY INDEX OF STATION SPECIES

SPECIES	STATION†																FREQUENCY INDEX (%)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
A. Herbaceous:																	
Actea alba						R							R			R	19
Adiantum pedatum			R														6
Amphicarpa bracteata (p)‡					R			C	C		R	A		R			38
Aralia nudicaulis (p)				C	R			R			C	C	R	C	C	R	56
Arisaema triphyllum (p)	R	C	C			C	R	C	C	C		C		R	R		69
Aster sp.						C		R	R		R	C	R		C	C	50
Carex pennsylvanica (p)				A	A	R					A	C	R	R	C		50
Caulophyllum thalictroides (p)	R	R		R			R	C	C	R					C		50
Circaea latifolia						C									C		13
Cryptotaenia canadensis						C											6
Cypripedium parviflorum											R						6
Desmodium strictum (p)				R							R	R	R		C	R	38
Dioscorea villosa	R							C	R		R	C	R				38
Eupatorium purpureum (p)						C			R	R					R	R	31
Eupatorium rugosum (p)			R	R				C	R						R	C	38
Galium sp.													R	R	R	R	25
Geranium maculatum (p)			R	R	R	C	R	C	R		R	C			C	C	69
Geum sp.							R										6
Hepatica acutiloba (p)						C		R	C		R	R	R	R			44
Helianthus sp.					R						R						13
Laportea canadensis (p)						R											6
Menispermum canadense (p)						C											6
Osmorhiza claytoni				R													6
Phryma leptostachya (p)						C											6
Podophyllum peltatum (p)	R	R	R			C	R	R	R	R	C	C	R			C	75
Polygonatum pubescens (p)			R	R	R			C	C					R	C		44
Prenanthes sp.	R			R								R					19
Ranunculus septentrionalis (p)									R		R			R			19
Sanguinaria canadensis			R					C						R			19
Sanicula sp.						R											6
Smilacina racemosa (p)		C	C		C		R	C	C	C	C	R	R	R	C	C	81
Smilax ecirrhata (p)	R	R	R					R	R		R				C		44
Solanum dulcamara (p)						R											6
Solidago latifolia (p)	R	R	C	C	R		C	C	C	C	C	C	C	R	C	C	94
Solidago sp.				R	R					R	R	C	R	R	C		50
Thalictrum dioicum (p)										R	R				C		19
Trillium grandiflorum						R		R					R				19
Trillium recurvatum									R								6
Uvularia grandiflora (p)						C	R	C	C	C	R			R	C		50
Viola sp.																C	6
No. of species per station	7	6	10	11	9	17	8	17	17	9	18	14	13	13	18	12	

* Relative abundance: A, abundant; C, common; R, rare.

† Stations 1–10 sampled in 1938; stations 11–16 sampled in 1939.

‡ (p), one or several plants or their root systems photographed in 1938–1940.

TABLE 1—*Continued*

Species	Station†																Frequency index (%)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
B. Woody:																	
Acer saccharum (p)	A	A	A	C	C	C	A	C	A	A	A	A	A	A	A	A	100
Amelanchier canadensis								C			R		C	C			25
Carya cordiformis			R		C	C	C		C				R	R	C	R	56
Cornus alternifolia			C									R			R		19
Fraxinus americana (p)	C		C	C	C	C		R	C	C	C	C	C	A	A	C	88
Fraxinus nigra								R			R						13
Hamamelis virginiana			R									C					13
Lonicera dioica (p)	C			C							C	C	A	R	R		44
Ostrya virginiana		C	R	C	C			C	C	C	C	C	C		C	C	75
Parthenocissus quinquefolia (p)			C	R	R	C	A	C	C	R	C	R	R		A		75
Prunus serotina	R		R	R							R			C			31
Prunus virginiana (p)	C	C	C	C			C		C	C		R	C	C	R	C	75
Quercus alga (p)		C	R	C	C	C	C	C	R		A	A	C	C	C	C	88
Quercus borealis maxima (p)	C	A	C	A	A	C	C	A	C	R	C	C	A	A	A	C	100
Rhus radicans																C	6
Ribes cynosbati													C	R			13
Tilia americana	C	C					C		C	C	C		A	C	C	C	63
Ulmus americana (p)		R							R			C			R		25
Ulmus fulva	C		R									C	C	C	C	C	44
Viburnum acerifolium				C	A					C	C	C	C		R	R	50
Vitis sp.											R					C	13
Zanthoxylum americanum	C	R					C				R		R				31
No. of species per station	9	8	12	10	8	6	8	8	10	8	14	13	15	12	14	12	

A detailed cover or frequency analysis of the vegetation was not made.[1] Table 1 shows the presence, relative abundance, and frequency index of species found, based on circular areas 25 yards in diameter around each trench.

The stations were designated numerically, since they could not be differentiated individually by any one characteristic. While it would be desirable to classify the sixteen stations on the basis of vegetation, it was impractical and confusing since their floristic composition was not sufficiently variable and other factors in the selection of a station were slighted unwarrantedly.

Investigation

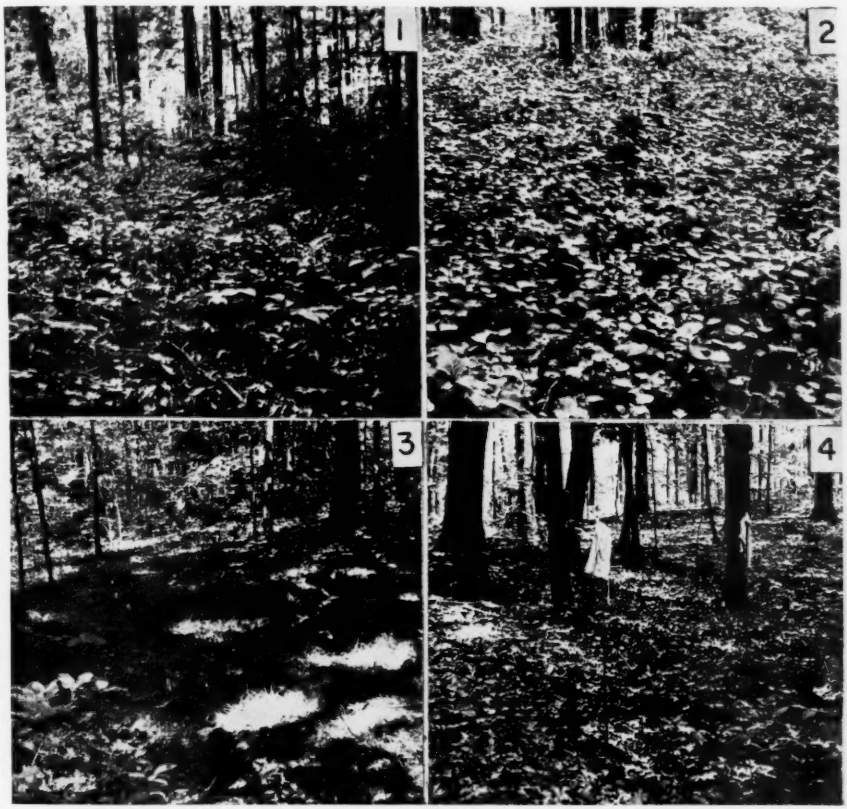
General methods

The sixteen stations were selected on the basis of relative abundance and type of herbaceous and woody ground cover, nature of soil, and topographical situation. A cross-sectional sample of conditions within the forest was thus obtained.

[1] Dr. Charles E. Olmsted, under whom this study was completed, has been conducting an investigation of the environment of this forest for a period of 6 years. The data, when published, will provide a treatment of the floristic composition.

Those station types which occupied the larger areas were sampled several times to assure reasonably accurate measurement of their root distribution and development (figs. 1–4).

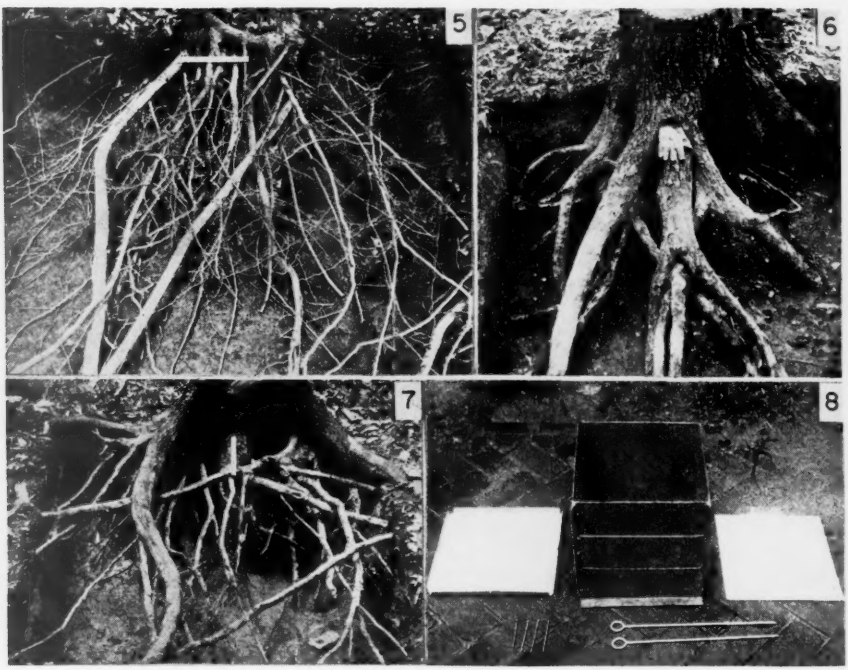
The methods used at stations 1–10 were, with some variations, similar to those initially employed by WEAVER (**18**) and those recently followed by TURNER (**16**),



FIGS. 1–4.—General views of some station types. Fig. 1, station 9; fig. 2, station 13; fig. 3, station 4; fig. 4, station 2.

BILLINGS (**2**), LUTZ, ELY, and LITTLE (**10**), and COILE (**3**). The rectangular trenches employed were $7\frac{1}{2} \times 2\frac{1}{2}$ feet. The area charted was 6 feet long horizontally, to the depth of the trench vertically, and on the wall nearest magnetic north. Stations 11–16 were sampled by a new method in which equal areas of all four walls of a square trench were charted. Such trenches were 4 feet square. The area charted on each wall was 3 feet long and to the depth of the trench (fig. 21).

After a station was chosen, either a rectangular or square trench was opened in a representative area sufficiently removed from trunks of any large trees and to a depth in the C horizon averaging 3–3½ feet, where the roots were significantly few in number. Vertical walls were maintained and roots projecting into the trench were cut off. After the wall or walls to be charted had been cleaned, the horizontal

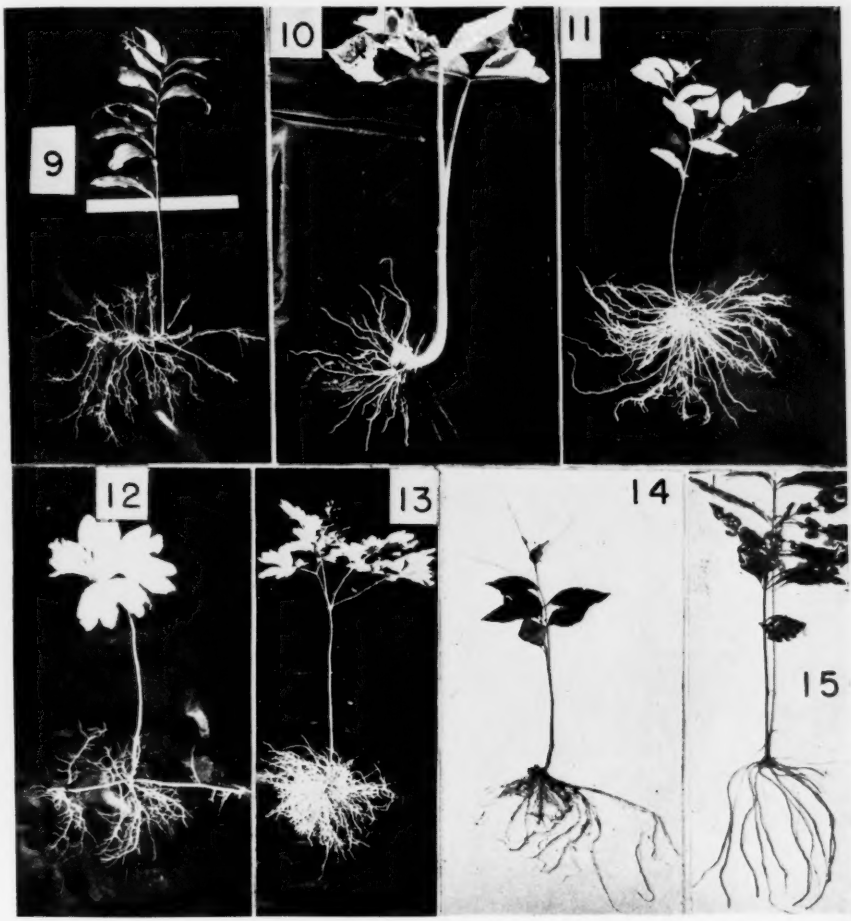


FIGS. 5–8.—Figs. 5–7, excavated specimens of dominant tree species: 5, *Acer saccharum;* 6, *Quercus alba;* 7, *Quercus borealis maxima.* Fig. 8, soil sample box for root-volume determinations.

boundaries of the profile were marked. Each area was ruled into square feet by horizontal and vertical strings. The horizons were charted on a scale of 3 inches to 1 foot.

A heavy, square-foot wire frame, divided into 2-inch squares by fine wire, was used in charting all profile inclusions. This was placed successively on each square foot of the trench face, which was carefully worked over with a fine pointed instrument to locate all root ends. Living, dead, woody, and herbaceous roots were then charted. Seven diameter size classes were recognized: 0–1, 1–2, 2–3, 3–4, 4–5, 5–10, and > 10 mm.

After charting the roots, the horizons were examined for other characteristics and possible inclusions. Counts were made of earthworms and cicada nymphs,

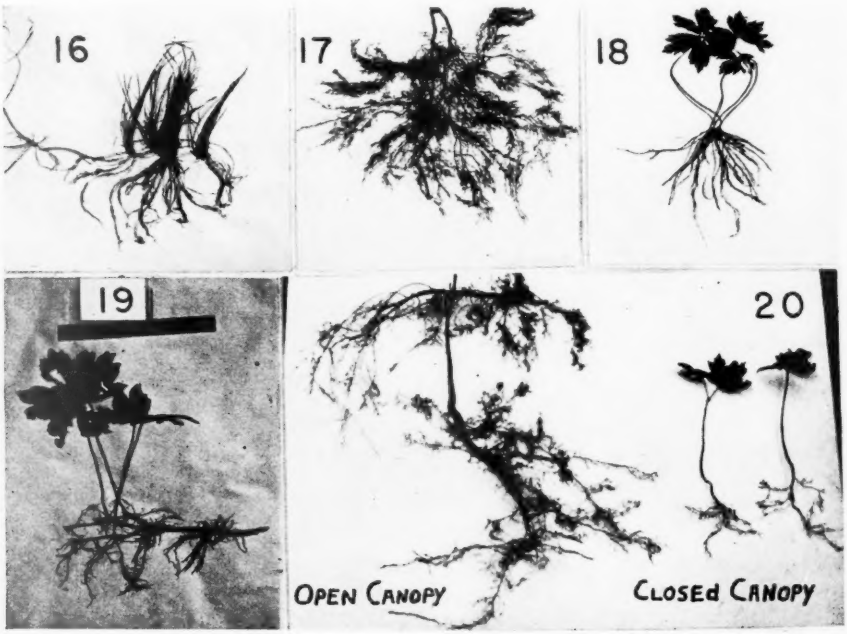


FIGS. 9–15.—Root systems of some native species. Fig. 9, *Smilacina racemosa;* fig. 10, *Arisaema triphyllum;* fig. 11, *Uvularia grandiflora;* fig. 12, *Podophyllum peltatum;* fig. 13, *Caulophyllum thalictroides;* fig. 14, *Phryma leptostachya;* fig. 15, *Eupatorium purpureum.*

and their burrows were recorded on the profile chart. The root systems of characteristic woody seedlings and herbaceous species, located either at or near the edge of a trench, were excavated and their relations to the surface horizons

noted. In other cases the soil was washed away from excavated blocks. The entire plants, or occasionally only their root systems, were photographed (figs. 9–20).

Root volumes were determined in the upper cubic foot of soil in different station types. For this purpose a soil-sample box was devised, permitting the subdivision



FIGS. 16–20.—Root systems of some native species. Fig. 16, *Carex* sp.; fig. 17, *Solanum dulcamara;* fig. 18, *Geranium maculatum;* fig. 19, *Parthenocissus quinquefolia;* fig. 20, *Acer saccharum* (1936 seedlings from open and closed canopies).

of a sample into three successive sections, each 4 inches in depth. The box was constructed of $\frac{1}{16}$-inch rolled-sheet steel welded to give an inside volume of 1 cubic foot. The bottom edges were beveled to make a sharp cutting edge. The plate used to divide the total sample into three units, 4 inches deep, was driven, guided by two slightly raised lips, through two narrow slots on one side of the box. By successive driving and digging around the box, an accurate cubic-foot sample, with its roots, could be obtained (fig. 8). Roots $1\frac{1}{2}$–2 inches in diameter were easily cut in preliminary sampling. Root volumes were measured by displacement in water.

Statistical methods

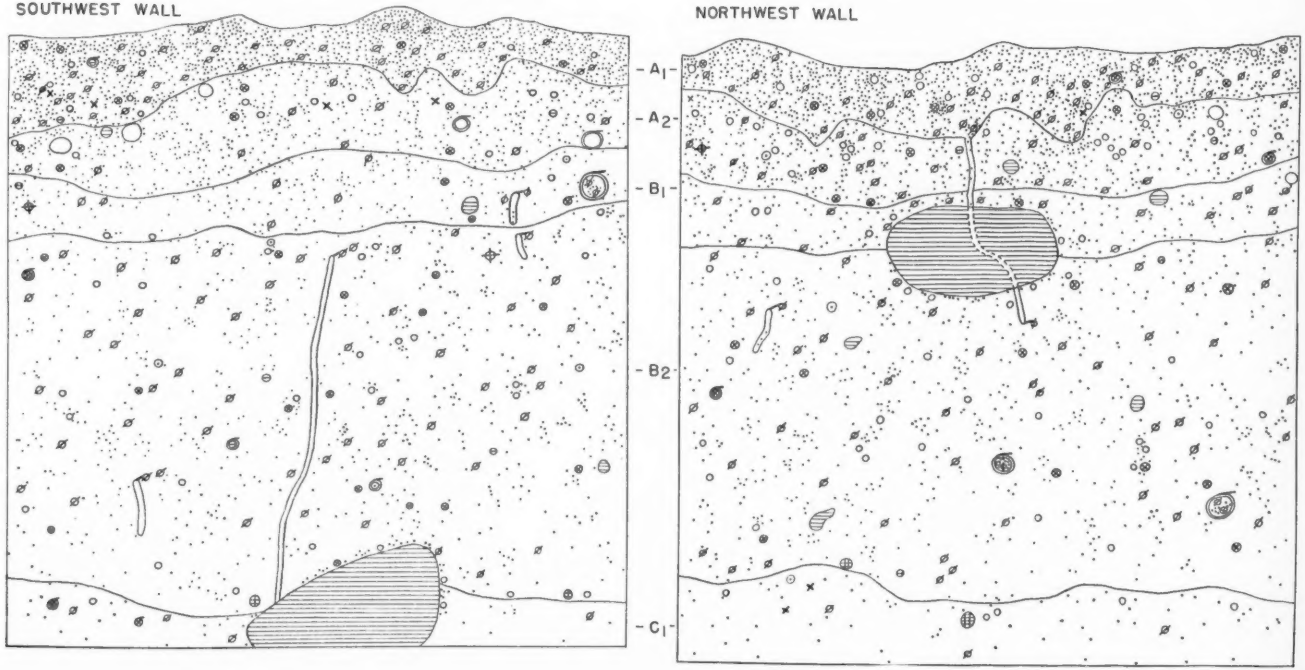
In measuring the degree of heterogeneity exhibited by the root distribution in the various station types, it was necessary to treat the data statistically. The standard deviation of means, that is, standard error (standard error $\sqrt{\frac{\Sigma V^2 - \frac{(\Sigma V)^2}{N}}{N(N-1)}}$, where Σ is the symbol for summation, V is the individual observation, and N is the number of variants) was obtained for each wall of square trenches as well as for total root data of the four walls.[2] Accordingly, the limits within which variations of the means occur because of uncontrollable conditions were defined. The standard error of the difference (standard error of difference = $(S.E._x)^2 - (S.E._y)^2$, where $S.E._x$ and $S.E._y$ are the standard errors of the means of x and y, respectively, was determined when two walls of the same square trench were compared. The t test for significance was used accordingly ($t = \frac{\overline{D}}{S.E._{\overline{D}}}$, where $\overline{D}$ is the difference between means and $S.E._{\overline{D}}$ is the standard error of the difference). The value t was used to determine the probability (P) according to the table for P values as given by Fisher (5). Differences not significant at the 0.05 level were not considered statistically significant (14).

Soil factors

The soil surface about station types with sparse ground cover (stations 1, 2, 3, 14, and 16) showed varying degrees of erosion, evidenced by the partial exposure of tree roots 2–3 inches in diameter and the occasional appearance of the A_2 and B_1 horizons. Judged by its typical grayish yellow color, the A_2 horizon was absent in portions of the profile sections in stations 7 and 9. In stations 4, 5, and 11, with ground cover predominately of sedge, all horizons were characterized by greater sandiness than at other stations.

In all profiles, the B_2 horizons (of heavy, reddish brown sandy clay) possessed the greatest depth and compactness. It typically showed many roots penetrating along the sutures formed by the irregular nut structure. The smallest roots (0.1–1.0 mm.) frequently were found penetrating the smaller soil blocks in the upper portions of the horizon, while this condition was relatively rare in the lower, more compact soil with larger blocks. Resistance to root penetration was evident from the flattened form of the smaller roots (< 2 mm.), when following the suture lines. Internal drainage is greatest along the sutures of the nut structure. It appeared comparatively favorable except in stations 1, 2, 3, 14, and 16, where greater

[2] Square trenches readily permit application of statistical methods. Accordingly, statistical treatment was confined to comparisons of variability in root numbers among station types sampled by such trenches and among their four walls (table 7).


SOUTHWEST WALL
NORTHWEST WALL
A1
A2
B1
B2
C1

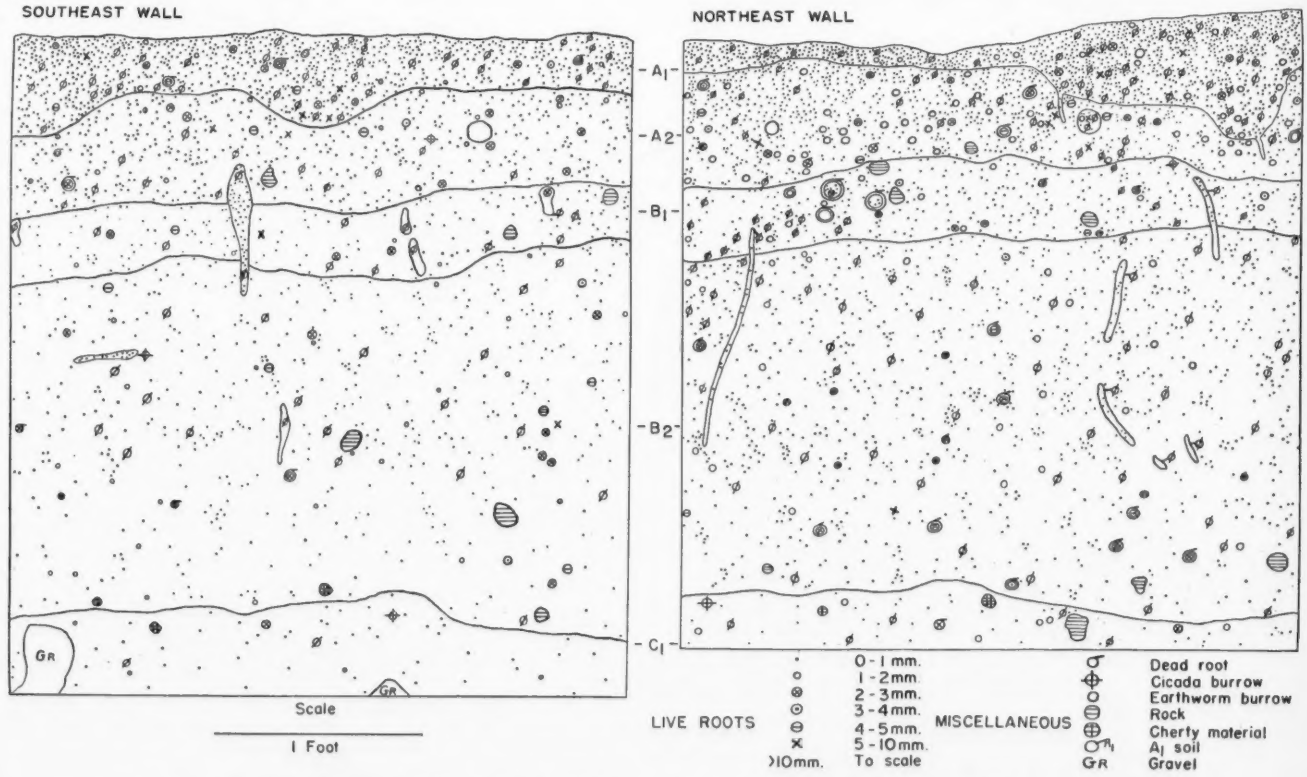


FIG. 21.—Root distribution and other horizonal inclusions in S.W., N.W., S.E., and N.E. profile walls of station 12; 1939

plasticity of the B_2, with marked increase in the clay content, interferes with percolation. Conversely, conditions for greatest drainage were found in stations 4, 5, and 11, with distinctly sandier profiles.

The surface soil of all stations exhibited numerous earthworm (*Lumbricus* spp.) middens (**6**) of variable diameter and height. Typically they were 4–5 inches wide and 1–1½ inches high. Cross-sections of some of the middens and accompanying burrows have shown leaf blades and petioles, in various stages of decomposition, to a depth of 6–8 inches. Burrows of smaller earthworms were also found. The larger burrows were lined with a film of dark organic soil which originated from worm castings; the burrows of the smaller earthworms had no visible organic soil lining. Apparently lining of burrows is accomplished over several years of active use. Middens play an important part in giving the surface soil an irregular local topography. Their black, highly organic soil withstands erosion very effectively. Some of the height of those located on slopes was developed by erosion about them.

Earthworms are the most conspicuous group of animals appearing in the profile sections. Greatest activity was manifest in the A_1 horizon, where they are largely responsible for the soil's porous nature. Numerous castings studded the middens proper and the soil surface at some distance from them. Burrows were found also throughout midden-less areas. The worms apparently play as important a role in mixing and aerating the surface horizons (A_1 and A_2) as they do in breaking down the leaf litter. In the latter case it is known that earthworms do eat leaf materials (**4**), yet their over-all effect upon breakdown of leaf litter probably centers about creation of a more favorable environment for bacteria and fungi through movement of leaves into the burrows.

In all profiles, regardless of the resistant nature of the B horizons, earthworms and their burrows were found in the C horizon at depths of 3–3½ feet, and in deeper trenches at 5 feet. The burrows were fairly perpendicular, with deviations largely where rocks blocked the path. No differences in depth of penetration were found in the profiles investigated.

The periodical cicada, *Magicicada septendecim* (**15**), was also conspicuous. The stage initially encountered in 1938 was the fifth instar or last stage prior to the adult. The former emerged during the spring of 1939. The varied nymph and pupal stages (**1, 12**) of the insect had penetrated the profile to depths of 3–3½ feet. In 1938 some nymphs were found in pockets which, approximating ½-inch in diameter and ¾–1 inch in height, just housed them. More typically this chamber extended upward several inches, a nymph lying at the bottom. In the latter cases the nymphs apparently had started their upward movement through the soil, but such burrows ended abruptly in either the B_2 or C_1 horizon. They were never found in the upper horizons (A_1, A_2, or B_1).

Burrows, formed by nymphs in the previous generation, had apparently been filled.[3] The trenches opened in 1939 revealed the burrows resulting from emergence of the nymphs. Mounds, built by the nymphs over the mouths of their burrows, were composed of B horizon soil, had an average basal diameter of 1 inch and a height of $1\frac{1}{2}$–$2\frac{1}{2}$ inches, with walls $\frac{3}{16}$–$\frac{4}{16}$ inch thick. They typically were confined to wooded areas where organic forest debris was abundant on the surface soil. In open areas outside the forest such mounds were rare.

Open cicada burrows, extending almost perpendicularly from their surface openings to varying depths, were common in all profiles investigated in 1939 and 1940. On 30-meter quadrats sampled at random, an average of fourteen cicada mounds per square meter was found.

In the A_2, B_1, and B_2 horizons of all profiles occurred varying-sized pockets of A_1 soil, from the filling-in of old root holes after root decay. Similar smaller pockets were apparently the result of earthworm activity, particularly in the A_2. The castings were conclusive evidence in differentiating these latter pockets from filled-in root holes, which also often contained fragmentary and undecomposed portions of roots. Some A_1 soil probably washes into earthworm burrows. Small herbaceous and woody roots often were found where, as a result of the union of several adjacent burrows, pockets of dark soil were developed with diameters of $\frac{1}{2}$ inch or more. This was also true in root holes which had been filled to the lower levels of the B_2 horizon. The concentration of roots in such pockets frequently approached, and occasionally equaled, that typical of the A_1 horizon. The roots in such pockets did not have the flattened form typical in the B_2. Some pockets of apparently A_1 soil in the B horizons, approximating $\frac{1}{2}$ inch in diameter, were probably the filled-in burrows of the cicada nymphs which emerged in 1922. Still larger pockets (6–8 inches in diameter) probably were filled-in burrows of rodents.

Root data

The basic data on root distribution (table 2) indicate the total number of live roots in the horizons of each station. The seven size classes recognized are differentiated into two groups, < 1 mm. and > 1 mm. The number of roots per square foot of horizon surface was greatest in horizons A and B, the former always with a much larger number than the latter. The number of roots per square foot of C horizon was low in all cases. The apparent relative suitability of horizons A_1, A_2, B_1, B_2, and C_1 for root development, as measured by numbers, decreased—with a few exceptions—in passing downward. With the exception of stations 5, 7, and 15, the greatest total decrease in root numbers per square foot of horizon surface occurred in passing from the A_1 to the A_2, although the latter was encountered

[3] Mr. William P. Longland, superintendent of Wychwood, stated that cicada nymphs had emerged in exceedingly large numbers in 1922. He judged the numbers in 1939 to be equally as great.

at the relatively slight average depth of 4 inches. These three stations showed marked exception to the typical root distribution.

TABLE 2

NUMBER OF LIVE ROOTS PER PROFILE HORIZON

Horizon	Total no. of roots	No. of roots per square foot of rock-free area		Total no. of roots	No. of roots per square foot of rock-free area		Total no. of roots	No. of roots per square foot of rock-free area		Total no. of roots	No. of roots per square foot of rock-free area	
		<1 mm.	>1 mm.		<1 mm.	>1 mm.		<1 mm.	>1 mm.		<1 mm.	>1 mm.
	Station 1*			Station 2			Station 3			Station 4		
A_1	460	116	31	591	450	23	1067	423	55	2072	1028	41
A_2	230	33	14	273	87	20	311	166	22	684	218	25
B_1	50	12	3	111	46	11	523	91	15	453	89	9
B_2	99	8	2	380	27	4	137	46	5	364	69	6
C_1	14	5	.....	13	7	2	166	33	1	403	55	6
	Station 5			Station 6			Station 7			Station 8		
A_1	1857	1289	6	2487	657	30	1185	526	16	1781	870	21
A_2	1495	740	71	436	222	11	451	649	29	797	213	11
B_1	315	130	14	383	138	8	785	461	23	375	172	13
B_2	965	68	6	644	116	7	1537	157	9	772	66	6
C_1	11	9	2	270	70	1	18	68	.....	65	32	3
	Station 9			Station 10			Station 11			Station 12		
A_1	1912	680	31	1873	868	22	3541	891	31	3897	1094	24
A_2	256	381	29	1060	353	21	2268	371	22	2510	464	28
B_1	405	195	11	379	225	18	862	216	10	927	252	12
B_2	1070	98	7	1074	93	7	3021	133	8	3009	136	8
C_1	180	107	8	181	91	3	431	57	4	220	65	7
	Station 13			Station 14			Station 15			Station 16		
A_1	4656	820	50	2043	554	28	1595	354	41	1666	453	57
A_2	1763	321	26	1281	207	15	1081	378	53	1004	129	18
B_1	721	211	11	438	177	11	362	161	17	416	114	16
B_2	1707	113	10	1695	87	9	1443	59	7	852	55	13
C_1	456	42	5	410	49	4	206	37	4	314	22	7

* Data for stations 1–10 based on one wall 6 feet long; for stations 11–16, on four walls totaling 12 feet long.

A concentration of the smallest roots, 0.1–1.0 mm. in diameter, was found in the A_1 horizon (table 2), indicating the location of much of the root water-absorbing

surface. This size class, per unit area, decreased markedly in the lower horizons. Contrary to possible expectations, the larger roots (> 1 mm.) showed no increase in numbers per unit horizon surface below the A. Further, within the A horizon only stations 5, 7, and 15 showed such an increase in the A_2 as compared with the

TABLE 3

ROOT NUMBERS AND PERCENTAGE ROOT AREA PER SQUARE FOOT OF ROCK-FREE HORIZON SURFACE*

Station	Root unit	Horizon				
		A_1	A_2	B_1	B_2	C_1
1	No.	147	47	15	10	5
	Area	1.913	0.341	0.025	0.029	0.001
2	No.	473	107	57	31	9
	Area	0.535	0.365	0.399	0.122	0.052
3	No.	478	188	106	51	34
	Area	1.291	0.217	0.119	0.012	0.004
4	No.	1069	243	98	75	61
	Area	0.676	0.276	0.162	0.025	0.096
5	No.	1295	811	144	74	11
	Area	0.441	1.459	0.243	0.031	0.006
6	No.	687	233	146	123	71
	Area	1.817	0.157	0.321	0.248	0.017
7	No.	542	678	484	166	68
	Area	0.275	0.407	0.584	1.023	0.001
8	No.	891	224	185	72	35
	Area	0.360	0.204	1.363	0.581	0.015
9	No.	711	410	205	105	115
	Area	0.813	0.138	1.360	0.447	0.108
10	No.	890	374	243	100	94
	Area	0.987	0.423	0.217	2.115	0.031
11	No.	922	293	226	141	61
	Area	0.723	0.555	0.195	0.121	0.064
12	No.	1118	492	264	144	72
	Area	0.758	1.078	0.301	0.099	0.075
13	No.	870	347	222	123	47
	Area	2.116	0.642	0.180	0.176	0.111
14	No.	582	272	188	96	53
	Area	0.948	0.391	0.218	0.093	0.064
15	No.	395	431	178	66	41
	Area	0.416	1.459	0.309	0.093	0.025
16	No.	510	147	130	68	29
	Area	0.647	0.221	0.259	0.240	0.257

* Here and in subsequent tables, profile data for stations 1–10 based on one wall 6 feet long; for stations 11–16, on four walls totaling 12 feet long.

A_1. Greatest numbers of dead roots were found in the lower horizons. The smaller roots (< 5 mm.) were found dead more frequently.

From the root distribution recorded on the charts in the field, the number of living roots and the percentage cross-sectional area occupied by them were calculated on three different bases for each station. These were (*a*) root numbers and percentage area per square foot of rock-free horizon surface (table 3); (*b*) root

numbers and percentage area per square foot of rock-free vertical profile sections occupying 0–1, 1–2, 2–3 foot depths (table 4); and (*c*) root numbers and percentage area per square foot of vertical profile sections 3 feet in depth (table 4). Root areas were obtained by use of the seven diameter size classes. Roots 0.1–1.0

TABLE 4

ROOT NUMBERS AND PERCENTAGE ROOT AREA PER SQUARE FOOT OF ROCK-FREE VERTICAL PROFILE SECTIONS 0–1, 1–2, 2–3, AND 0–3 FEET DEEP

Station	Depth (feet)	Root no.	Root area (%)	Station	Depth (feet)	Root no.	Root area (%)
1	0–1	96	1.191	9	0–1	444	0.361
	1–2	15	0.047		1–2	105	0.059
	2–3	10	0.029		2–3	79	0.035
	0–3	41	0.422		0–3	209	0.152
2	0–1	163	0.438	10	0–1	525	0.255
	1–2	41	0.139		1–2	130	0.199
	2–3	23	0.065		2–3	123	0.178
	0–3	76	0.214		0–3	259	0.211
3	0–1	275	0.606	11	0–1	528	0.547
	1–2	60	0.030		1–2	179	0.157
	2–3	25	0.003		2–3	96	0.114
	0–3	120	0.214		0–3	268	0.273
4	0–1	477	0.386	12	0–1	603	0.767
	1–2	88	0.097		1–2	174	0.167
	2–3	82	0.094		2–3	95	0.084
	0–3	216	0.192		0–3	291	0.339
5	0–1	607	0.865	13	0–1	573	1.249
	1–2	99	0.032		1–2	137	0.113
	2–3	48	0.028		2–3	65	0.101
	0–3	251	0.308		0–3	258	0.488
6	0–1	501	0.359	14	0–1	330	0.501
	1–2	135	0.067		1–2	98	0.238
	2–3	84	0.005		2–3	55	0.112
	0–3	240	0.143		0–3	161	0.284
7	0–1	486	0.305	15	0–1	284	0.572
	1–2	143	0.064		1–2	62	0.081
	2–3	55	0.012		2–3	40	0.041
	0–3	228	0.127		0–3	129	0.231
8	0–1	467	0.213	16	0–1	233	0.307
	1–2	113	0.208		1–2	76	0.213
	2–3	48	0.024		2–3	34	0.342
	0–3	210	0.148		0–3	114	0.287

mm. were assigned diameters of 0.75 mm. Exact diameters of roots > 10 mm. were obtained in the field. Accepted diameters of the five remaining size classes were the midpoints of their ranges.

Based on root numbers and percentage root area per square foot, the suitability for root growth of the specific horizons A_1, A_2, B_1, B_2, and C_1 was variable. Twelve stations showed both greater root numbers and higher percentage root area in the A_1 than in the A_2. Of the other stations, only two—stations 7 and 15—showed both greater root numbers and area in the A_2 than in the A_1. The latter stations were

selected because a dense and homogeneous layer of *Parthenocissus quinquefolia* dominated the ground cover of both areas. The roots of this species are found almost exclusively in the upper portions of the A_2 horizon. In station 5 there was a higher percentage of root area in the A_2 horizon but not a greater number of roots. Here the difference coincided with the association of a large number of small shrubs with comparatively homogeneous patches of sedge. In no station did the B_1 horizon show both greater root numbers and percentage root area than did the A_2. This was true successively in the B_2 and C_1, where the influence of large roots upon the calculated area gave higher values that were not significant. In terms of both root area and numbers, however, the B_2 and C_1 horizons exhibited the least suitability for root development and showed less variation from the one to the other than did the upper horizons. These general statements, with the few exceptions, hold true regardless of the peculiarities of the different stations.

The greater total and percentage decreases of both root number and area, in profile sections 0–1, 1–2, and 2–3 feet in depth (table 4), occurred in passing into the second foot. The decrease was less from the second to the third foot. Station 8 decreased more in area in passing from the second to the third foot but showed typical decreases in root numbers with increased depth. The greatest root area in station 16 occurred in the third-foot section. These two stations, however, showed greater total and percentage decrease of root numbers from the first to the second foot level. Generally, the larger the root numbers and total area in the top sections, the greater the percentage decrease at the second foot level. Root frequency definitely decreases with increased depth.

Profile areas 3 feet in depth (table 4) at stations 1, 2, 3, 14, and 16, selected because of dearth of ground cover, were characterized by comparatively large percentage root areas and small root numbers, that is, few but large roots. Stations 4–13 and 15, with abundant ground cover of varied floristic composition, were characterized by large root numbers and small average root areas (table 2). Station 15, an exception to this general condition, had an average area per root approximately equal to that of station 14, which possessed the smallest average area per root of the five "bare" stations. This can be explained in part by the association of a number of woody species (table 1) with the society of *Parthenocissus* sampled at the former station, influencing the number of roots > 1 mm.

The number of earthworm burrows per square foot of profile areas indicated great activity in all station types. Such burrows occurred less frequently in stations where patches of sedge were associated with sandier silt loam soils. The greatest number of cicada pupae and burrows were found where red oaks were dominant in the canopy, but the nymphs and their burrows were found in all the trenches (table 6). Such burrows afforded significant drainage during rains heavy enough to yield run-off.

The total volume of roots in an upper cubic foot of soil was variable throughout the station types (table 5). Areas with sparse ground cover yielded root volumes equal to those of areas with abundant ground cover. In samples which contained no roots > $\frac{1}{2}$ inch in diameter, root volume decreased successively in the 0–4, 4–8,

TABLE 5

VOLUME OF ROOTS IN ONE CUBIC-FOOT SOIL BLOCKS SUBDIVIDED INTO 4×12×12 INCH SECTIONS

Sample	Ground cover type	Depth of soil block section (inches)	Percentage root volume	
			Per soil block section (4×12×12 inch)	In total cubic foot
1	Mixed mesic spp.	0–4	0.481	0.261
		4–8	0.167	
		8–12	0.136	
2	Mixed mesic spp.	0–4	0.659	0.453
		4–8	0.628	
		8–12	0.073	
3	Bare	0–4	0.963	2.164
		4–8	4.272	
		8–12	1.256	
4	Bare	0–4	0.235	0.535
		4–8	1.361	
		8–12	0.010	
5	C. pennsylvanica society	0–4	1.717	1.148
		4–8	1.549	
		8–12	0.178	
6	C. pennsylvanica society	0–4	2.795	2.494
		4–8	4.550	
		8–12	0.136	
7	Lonicera dioica society	0–4	1.413	0.530
		4–8	0.157	
		8–12	0.020	
8	Lonicera dioica society	0–4	1.895	0.813
		4–8	0.471	
		8–12	0.073	
9	Parthenocissus sp. society	0–4	0.701	0.656
		4–8	1.151	
		8–12	0.115	
10	Parthenocissus sp. society	0–4	1.266	0.484
		4–8	0.104	
		8–12	0.083	

and 8–12 inch levels. When the sample contained larger roots, the volume frequently was largest in the 4–8 inch level; but the 8–12 inch section never surpassed the 4–8 inch level. In all samples the 0–8 inch section contained 80 per cent or more of the total root volume.

The root systems of typical herbaceous and woody seedlings predominately

were confined to the upper 6 inches of the profile (figs. 9–20). Exceptions occurred in areas where openings in the canopy have existed for several years. There most root systems extended to depths of 12–18 inches.

The root systems of the selected specimens of the three dominant species of the forest—sugar maple, red oak, and white oak—were investigated in 1940 (figs. 5–7). The white oak, 223 years old, 23 inches in diameter at a height of 2 feet, was wind-

TABLE 6

COMPARATIVE STATION TYPE DATA

Station	Physiography		Depth to C$_1$ horizon (inches)	Important species of ground vegetation	No. of species with 50–100% frequency index		Earthworm burrows per square foot	Cicada burrows per total profile
	Character of topography	Elevation (feet)			Herbaceous (11 species recorded)	Woody (10 species recorded)		
1...	Southeast slope, 2.5°	961	36–42	Mesic woodland herbs and shrubs (rare)	4	5	14	3
5...	South slope, 4.5°	1019	36–43	Carex pennsylvanica (abundant)	6	8	8	3
7...	Southeast slope, 7.5°	1017	32–38	Parthenocissus quinquefolia (abundant)	7	7	7	6
8...	South slope, 4.5°	909	36–40	Mesic woodland herbs and shrubs (abundant)	9	6	9	3
11...	North slope, 3.5°	1011	29–36	C. pennsylvanica (abundant)	9	8	9	15
12...	Northwest slope, 2.5°	961	33–40	Mesic woodland herbs and shrubs (abundant)	9	8	17	9
13...	Northwest slope, 13°	1009	25–30	Lonicera dioica (abundant)	7	10	12	10
14...	Northeast slope, 4.5°	998	28–33	Mesic woodland herbs and shrubs (rare)	7	7	9	10
15...	South slope, 2.5°	972	29–35	P. quinquefolia (abundant)	10	10	21	4
16...	Southeast slope, 7°	951	30–35	Mesic woodland herbs and shrubs (rare)	8	9	14	4

broken in 1938. Its root system was the most massive of the three species. Typically deep rooted, it proportionately contributed a smaller amount of root area in the upper A_1, A_2, and B_1 than did the specimens of red oak and sugar maple studied. The red oak partially excavated was 97 years old, 22 inches in diameter at a height of 2 feet, and was also wind-broken in 1938. Its root system was less massive than that of the white oak but contributed heavily in both large and small roots to the upper three horizons. No large sugar maple was available for excavation, but a smaller specimen, wind-broken in 1940, was investigated. It consisted of two

sprouts, each 15 years old, and had a diameter of 4 and $3\frac{1}{2}$ inches, respectively, at a height of 2 feet. The most marked character of the root system was the mass of smaller roots ($< \frac{1}{4}$ inch in diameter) which ramified throughout the upper three horizons (fig. 5). The relative number of deep anchorage roots was as great as in the red and white oaks. The response of maple seedlings to the effects of increased insolation following tree wind-fall or wind-break and subsequent canopy opening was also of interest (fig. 20).

Discussion

The significance of some of the findings has been treated in sections on soil factors and root data. On the whole, the results are in accord with those of previous investigators. COILE (3) examined four profiles in an uneven-aged stand of white oak–black oak–red oak on Georgeville stony clay in the Duke Forest, North Carolina. KORSTIAN and COILE (9), in reporting the same study, state: "The most striking features are the relatively large number of small roots in the upper few inches of soil and the paucity of roots of the two smallest size classes ($<$ 0.1 and 0.11 to 0.30 inch) in the B and C horizons." While this condition is also true at Wychwood for comparable size classes (table 2), the present profiles, regardless of type of trench employed, showed greater numbers of roots in all horizons than were encountered by COILE. The only profiles comparable with his in number of roots per horizonal square foot were those here investigated because of scarcity of herbaceous and woody ground cover (stations 1, 2, 3, 14, and 16). The differences in root numbers probably are due to the regional locations of the respective stands and to differences in soil types and amount of herbaceous ground cover.

The soil type at Wychwood, Bellefontaine silt loam, obviously is capable of allowing the development of comparatively large numbers of roots, particularly in the A_1 and A_2 horizons. In the B_2 horizon the roots were found to penetrate the soil in comparatively large numbers, not only along the sutures of the characteristic nut structure, but also—though less frequently—through the nutlets.

In accordance with earlier findings (**16, 2, 10, 3, 13,** and others), this study has shown a differential suitability for root development in the different horizons. The greater number and percentage cross-sectional root area per square foot in the A_1 horizon was expected, for it is here that oxygen, water, and nutrient supplies are usually most favorable. The exception in stations 7 and 15, in which the A_2 surpasses the A_1 in numbers and root area, indicates that regardless of the potential suitability of a horizon for root development, the actual root distribution is conditioned by the type of root system of the dominant or subdominant species in the area. Further, root systems of such ground cover species as *Parthenocissus quinquefolia* will distort the results in any study where an attempt is made to measure only tree roots, unless great care is taken to identify each woody root encountered, particularly smaller ones. Especially is this true in deciduous forests, where

herb and shrub ground cover makes it almost impossible to obtain an accurate measure of tree roots only.

The concentration of root numbers and cross-sectional root area in the upper foot of the profiles suggests that aeration is possibly of significance. This point was further emphasized by the low number of smaller roots in lower levels of the profiles. Correspondingly there were more dead roots in the lower depths. While such findings point to lack of aeration as a limiting factor for root development with increased depth, it is probably not the only factor involved. This view is substantiated in areas where the canopy has been opened through wind-break or wind-fall, where tree seedling and herbaceous root development is greater at all levels of the profile. Thus it is debatable whether the greater development of roots in the upper part of the soil is a response to more favorable aeration, moisture, or temperature conditions. These factors, as well as a more abundant supply of available nutrients and inherent tendencies of native species when grown under low light intensity, probably are interrelated in influencing the greater root development in the upper soil section.

Areas comparatively free of ground vegetation—for example, stations 1, 2, 3, 14, and 16—had fewer roots although they possessed a higher average cross-sectional area per root than did those stations with abundant ground cover, regardless of type. The findings in the former stations thus primarily indicate the root condition of the trees which make up the canopy. On the other hand, root numbers recorded for profiles of "bare" stations are in themselves comparatively large. An area with abundant ground cover was found (station 15) whose root numbers and area equaled that of a "bare" area. This suggests that the relative proportion of woody to herbaceous roots fluctuates throughout the forest. It is possible that the woody roots in bare areas may draw heavily upon the supplies of the soil environment and thereby limit the establishment of herbaceous species. Unpublished results of trenched quadrat studies, however, suggest that such root competition is not critical.

When stations 1–10 were sampled during the summer of 1938 by rectangular trenches, it seemed that the selected wall was not always typical of the root distribution on the four walls. This was particularly true in areas with abundant ground cover where several herbaceous or woody plants appeared near one or more of the walls. Because of this visible local heterogeneity in root distribution, it was decided to test the usefulness of square trenches—in which equal areas on all four walls are charted. Such sampling should diminish the influence of root systems which, with predominating numbers, approach the trench from one direction. Further, since the problem of sampling root distribution is basically statistical, the method should yield a more accurate measure of the distribution with respect to all directions, especially where undue directional effects are evident. In the square trenches the root data from the four walls may be totaled.

In table 7, the two walls of each trench varying the most with respect to the A_1 horizon are compared statistically as to root numbers. Results much more significant were obtained when horizon extremes of each trench were compared. However, since only one wall would have been selected in using a rectangular trench, the comparisons are on a wall-for-wall basis.

The probability of occurrence for differences between two walls of the same trench ranged from 1 chance in 100 (very significant) to 1 chance in 2 (no significance). In station 12, located on a 2.5° slope, the two walls showed significant differences in the upper four horizons. Stations 11, 13, 14, 15, and 16 had walls

TABLE 7

t TEST OF ROOT NUMBER MEAN DIFFERENCES BETWEEN WALLS OF SQUARE TRENCHES

Station	Walls	Horizon	$\bar{D}/S.E._{\bar{D}}$	Value of t	Station	Walls	Horizon	$\bar{D}/S.E._{\bar{D}}$	Value of t
11	N.W. vs. N.E.	A_1	517/157	3.292*	14	E. vs. W.	A_1	209/69	3.028*
		A_2	136/42	3.238*			A_2	44/13	3.384*
		B_1	45/36	1.250			B_1	64/36	1.777
		B_2	5/12	0.416			B_2	13/5	2.600
		C_1	24/8	3.000*			C_1	8/6	1.333
12	S.E. vs. N.E.	A_1	564/93	6.064†	15	E. vs. S.	A_1	126/45	2.800*
		A_2	337/85	3.964*			A_2	166/109	1.495
		B_1	122/15	8.133†			B_1	84/28	3.000*
		B_2	85/15	5.666*			B_2	3/4	0.750
		C_1	24/11	2.181			C_1	8/11	0.727
13	N.W. vs. S.E.	A_1	388/124	3.129*	16	S.E. vs. N.W.	A_1	240/67	3.583*
		A_2	144/42	3.427*			A_2	41/29	1.413
		B_1	6/9	0.666			B_1	59/33	1.484
		B_2	36/15	2.400			B_2	14/13	1.076
		C_1	8/6	1.333			C_1	10/4	2.500

* Significant at 5% level.
† Significant at 1% level.

whose horizonal differences in root numbers were significant in 1 to 3 of the total five horizons. Two rectangular trenches were tested similarly. Here each initial 6-foot section was divided into two sections 3 feet in length to afford a statistical basis for application of the *t* test. In each of these stations the two sections differed significantly only in the A_2 horizon. In these cases either the writer was fortunate in selecting walls which showed little variation or it is to be expected normally that, owing to directional effects, one wall will show less variability than that between any two walls of the same trench. It is possible that the remaining three walls of these rectangular trenches would have shown significant differences from the selected 6-foot walls.

The results from the two types of trenches have been reported together, but the square trenches were assigned more weight. Root numbers obtained from rec-

tangular trenches in bare areas were comparatively lower than those obtained from square trenches in similar areas. Numbers secured from the former trenches were probably not an accurate sample of the true root distribution. The same is true to varying degrees of other station types investigated by the two types of trenches; for example, station 7 vs. station 15, station 5 vs. station 11, etc. The results from square trenches were obviously most accurate when listed as a total of the four walls, yielding a sample 12 feet in length compared with 6 feet for the rectangular trench.

The use of square trenches affords a basis for statistical comparison of root numbers obtained in different station types within the same plant community.[4] Observation of the basic differences of root distribution within a community on one soil type speeds the determination of the significant relationships of the same community on several soil types. The comparison may be focused on horizons, profile sections, or total profiles. Analysis of horizonal differences in root numbers between the different station types was carried out for stations 11–16.

Significant horizonal differences were obtained for stations 11, 12, and 13, with good ground cover, as opposed to stations 14 and 16, with sparse cover. Further, there were no horizonal differences in root numbers among stations 11, 12, and 13, or among stations 14 and 16, except in the C_1 horizon in the latter stations.[5] Such local differences might be expected considering the heterogeneous distribution of the dominant tree species, with their differing types of root systems. On the other hand, station 15, with a dense society of *P. quinquefolia* as ground cover, differed in the same direction from stations 11, 12, and 13 as did bare stations 14 and 16. However, the A_2 horizon of station 15 significantly surpassed the A_2 of the two bare stations in root numbers and was equivalent in numbers to the others with abundant cover. If one assigns to the A_1 and A_2 horizons more importance than to the others, station 15 must be segregated as possessing a distinct type of root distribution. This is in accord with the distribution in the A_1 and A_2 of this station, which is conditioned by the type of root system of the dominant ground cover species and not by the apparent relative suitability of the respective horizons.

The root-volume results give further evidence of the heterogeneity of root distribution in the deciduous forest. They indicate that the upper 8 inches of the soil contain the largest volume of roots. Samples were not taken below the first foot, but it seems unlikely that greater volumes would be found at lower levels—unless exceptionally large tree roots were encountered. The amounts are approximately equal to those found by WEAVER and HARMON (**19**) in the prairie.

[4] A standard error can be obtained for each trench, since each wall, by subdivision into 1-foot sections, can yield a mean observation.

[5] In the *t* test, "difference" is intended to mean only significant differences (those at or above the 5 per cent level in FISHER's probability table).

Earthworms, in mixing and penetrating all horizons, are obviously of benefit to the aeration, fertility, and water relations of the profiles. Such activity warrants further investigation. Cicada activity within the soil, while most vigorous during emergence of the nymphs, is favorable in providing drainage channels and in effecting better aeration, particularly of the B_2 horizon. Its effect is probably felt for a number of years in ungrazed forests.

Summary

1. The relationship of the Bellefontaine silt loam soil profile, with its inclusions, to the root development of the native species in the maple-oak forest at Wychwood, Lake Geneva, Wisconsin, was studied during the summers of 1938–1940. Stations were selected to obtain a cross-section of the varying conditions in the forest.

2. Sixteen station types were investigated by the trench method of root-distribution sampling. In six of the station types a new, square method of trenching afforded more accurate measurement of the heterogeneous root distribution than did the rectangular trenches most commonly employed.

3. Root systems of herbaceous and woody seedlings in the various station types were excavated and their relation to the upper horizons noted. Large specimens of sugar maple, red oak, and white oak also were partially excavated. Root volumes were obtained from surface cubic-foot samples in each station type. Cicada and earthworm activity was observed.

4. Greatest concentration of the smallest roots, 0.1–1.0 mm. in diameter, was in the A_1 horizon. Larger roots did not show an increase in numbers per unit horizon surface below the A horizons. Greater numbers of dead roots occurred in lower horizons.

5. On root number and percentage root-area basis, horizons A_1, A_2, B_1, B_2, and C_1 generally showed successive decrease in suitability for root development. On the same basis there were marked decreases, with two exceptions, of root numbers and area with successive 1-foot increases in depth to 3 feet.

6. Areas with sparse ground cover showed low root numbers and comparatively high percentage root areas. Stations with more abundant ground cover were characterized by larger root numbers and smaller percentage root areas.

7. Earthworm and cicada activities, particularly the former, were important factors in the soil environment. Such activities were more or less uniform throughout the woodland.

8. In all cubic-foot root-volume samples, the 0–8 inch level contained 80 per cent or more of the total root volume present in the sample. When the sample lacked roots $> \frac{1}{2}$ inch in diameter there were successive decreases in volume in passing into the 0–4, 4–8, and 8–12 inch levels.

This study was carried out under the supervision of Dr. Charles E. Olmsted, who suggested the problem. The writer expresses sincere appreciation to him for his interest and helpful suggestions. He is also indebted to Dr. Sewall Wright for advice on the statistical treatment of root data.

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FLORAL INITIATION IN BILOXI SOYBEAN AS INFLUENCED BY GRAFTING

P. H. HEINZE,[1] M. W. PARKER,[2] AND H. A BORTHWICK[3]

(WITH THREE FIGURES)

Introduction

There are wide differences among soybean varieties in the lengths of daily photoperiod under which they initiate flower primordia. Plants of the Biloxi variety subjected to daily photoperiods of 14 or fewer hours produce abundant flower primordia within a few weeks, but on photoperiods of 16 or more hours they do not produce sufficient flower-forming stimulus to cause floral initiation until they are many months old. The plants of several other varieties regularly initiate flower primordia when only a few weeks old, even though they are subjected to continuous light from the time they emerge from the soil (**2**). Some of these varieties do not continue the development of their flower primordia under continuous light, but others produce normal flowers and fruits. Batorawka and Agate are examples of this latter type.

Although these wide differences in photoperiodic response exist among certain varieties of soybeans, it seems probable that the basic mechanism causing floral initiation in the different varieties is fundamentally the same. Evidence supporting this assumption has been obtained by grafting Batorawka or Agate soybeans to Biloxi.

Literature review

Grafting has been employed as a means to study the photoperiodic response of plants by CAJLACHJAN (**4**), MOSKOV (**11, 12**), MELCHERS (**9**), MELCHERS and LANG (**10**), KUIJPER and WIERSUM (**7**), KUIJPER and SCHUURMAN (**8**), HAMNER and BONNER (**6**), and others. A general review of most of these papers has been made recently by CHOLODNY (**5**). A primary purpose in most of the studies has been to demonstrate that a stimulus causing flowering in one plant may cross a graft union and induce flowering in the other. In some experiments the two plants concerned have been of the same variety; in others they have been of different varieties, species, or even genera. Several workers have sought to show further that the flower-inducing stimulus of the one kind of plant is identical to or closely similar to that in the other. All the investigators just cited consider flowering to be a result of hormonal action.

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Only two of the papers mentioned (**7, 8**) deal with the grafting of soybeans. In these, plants that had been grown continuously on long photoperiods and were apparently vegetative were grafted with others that had formed flower buds as a result of treatment with short photoperiods just prior to grafting.

In the first paper (**7**) results are reported from twelve grafts. In six of these the scion was from the short-day plant and the stock from the long-day one; in the other six this arrangement was reversed. All the six long-day stocks produced visible flower buds or flowers, but only one long-day scion produced flower buds before the close of the experiment. No control grafts were included. The second paper (**8**) reports results obtained from thirty-eight successful grafts between plants grown on long and on short day, respectively, and from twenty controls in which stock and scion were both from plants grown on long photoperiod. Flower-bud formation on the long-day component of the grafted plants occurred in only five grafts, all of which were short-day scion on long-day stock.

In both these papers two varieties—Ked. 29 from Buitenzorg and a yellow-seeded variety from Vilmorin—were used. All the twelve grafts in which the long-day component flowered were of the Vilmorin variety. In eleven of these twelve, the long-day component was the stock, while in the other one it was the scion. From this the investigators concluded that the stimulus moved downward more readily than upward across the graft union. The results of this research indicate that flowering of a soybean grown on long day may be somewhat accelerated by grafting to it a part of a plant that has been subjected to short photoperiods. The data do not show, however, that flower primordia were not present on the long-day stocks when the plants were grafted.

Plants of all the yellow-seeded lots from Vilmorin have flowered earlier than varieties such as Mandell and Minsoy when tested at Arlington, Virginia. The various lots obtained from Buitenzorg, on the other hand, have been even later than Biloxi. The varieties Mandell and Minsoy have been found to initiate flower primordia under conditions of continuous light, but the flower buds develop very slowly on long photoperiod and seldom become visible (**2**). From this it seems probable that flower primordia were present on the Vilmorin plants used by KUIJPER, WIERSUM, and SCHUURMAN, and that the earlier flowering after grafting resulted from accelerated development of such pre-existing flower primordia. Failure of flowers to form on the plants of the Ked. 29 variety following grafting would indicate that primordia were not present on the long-day components previous to grafting and were probably not formed on them as a result of it.

Material and methods

Three varieties of soybeans (*Soja max*) and four varieties of garden beans (*Phaseolus vulgaris*) were used in these experiments. The soybean varieties were

Biloxi, Agate, and Batorawka. The garden bean varieties, all of which flower profusely even on long photoperiods, were Red Kidney, Plentiful, Black Valentine, and Dwarf Horticulture. All grafts included a Biloxi component that received long photoperiods both before and after grafting. This component was used to determine whether or not a flower-inducing stimulus crossed the graft union and

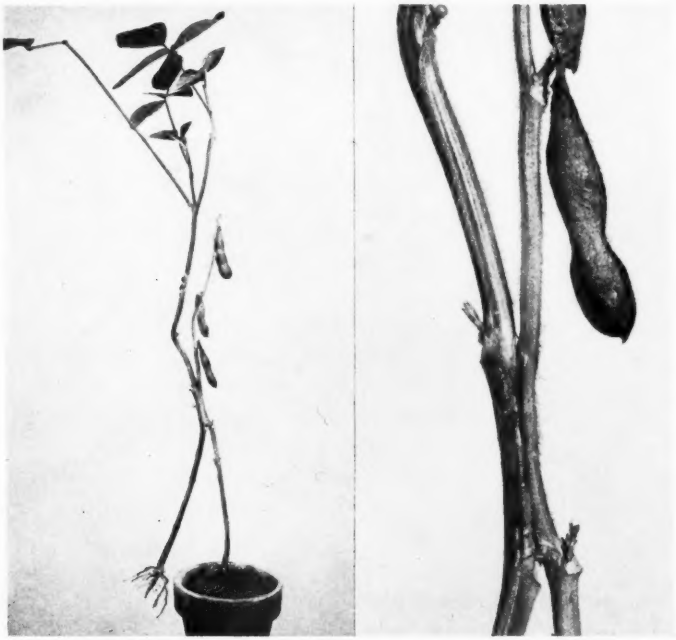

FIG. 1.—Left: approach graft of Biloxi and Agate soybeans. Right: detailed view of graft union after removal of Parafilm. Photographed about 2½ months after graft was made. Biloxi receptor initiated flower primordia soon after the grafting. A few of its flower buds finally opened but it developed no pods. Roots were removed from soil about 2 months before it was photographed.

is referred to as the receptor, following the usage of HAMNER and BONNER (6). In many of the grafts the receptor was combined with other soy or garden bean varieties, but in a few it was combined with another Biloxi plant that received short-day treatment either prior to or subsequent to grafting.

All plants were grown in 4-inch pots in the greenhouse, and on photoperiods of 17 or more hours, depending on the season. The photoperiods consisted of natural light plus Mazda light of about 50 foot-candles from sundown until midnight.

Several methods of grafting were used, including approach grafting of stems,

splice grafting of petioles and of stems, and bud grafting. The youngest plants in any experiment were 19 days old when grafted, and in no experiment were grafts made with plants older than 43 days. Approach grafts of two stems were made by removing a thin section 1–1½ inches long from adjacent sides of two stems. These cut surfaces were immediately placed against each other and wrapped with a rub-

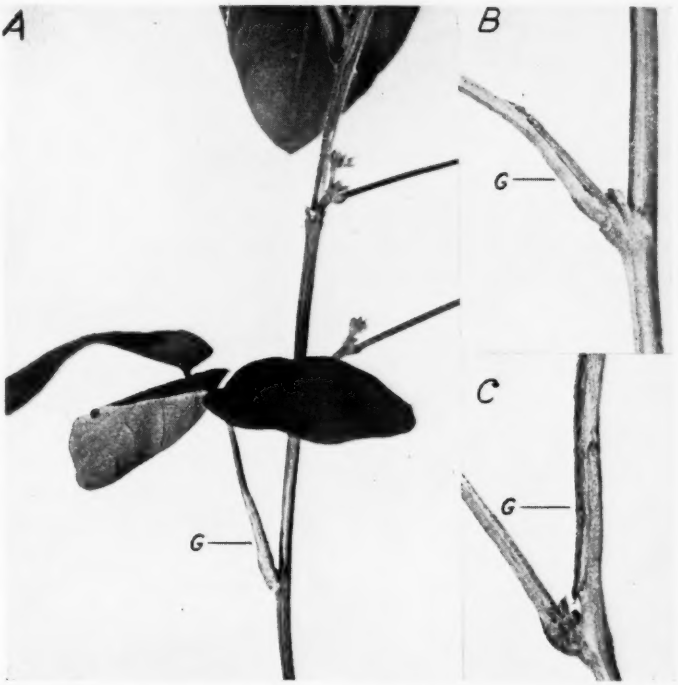

FIG. 2.—Splice grafts of petioles and of stems. *A*, Agate leaf grafted to Biloxi receptor; graft union wrapped with Parafilm. *B*, detailed view of petiole-graft union. *C*, detailed view of stem-graft union (*G*). Photographed 6 weeks after grafting.

berized paraffin tape known as Parafilm. The tape held the grafts firmly together but stretched easily as the stems increased in size (fig. 1).

In the petiole-grafting experiments, only one leaf was grafted to a single receptor plant. This leaf was always a young one and was in about the same stage of development as the leaf removed from the receptor. The petioles of the leaves were usually 1–2 inches long and the leaflets were about half their usual mature size. Both leaves were severed by slanting cuts about ¾ inch long made through the petiole, the lower end of the cut beginning on the under side of the petiole

near its base. The petioles were brought together along their cut surfaces and in most cases were bound with Parafilm (fig. 2*A*, *B*). The cuts were always made with a wet razor, and the surfaces were kept moist until the grafting operation was completed. In a few cases glass capillary tubing was used instead of the tape to hold the grafted parts in place. Immediately after the leaf was grafted, the

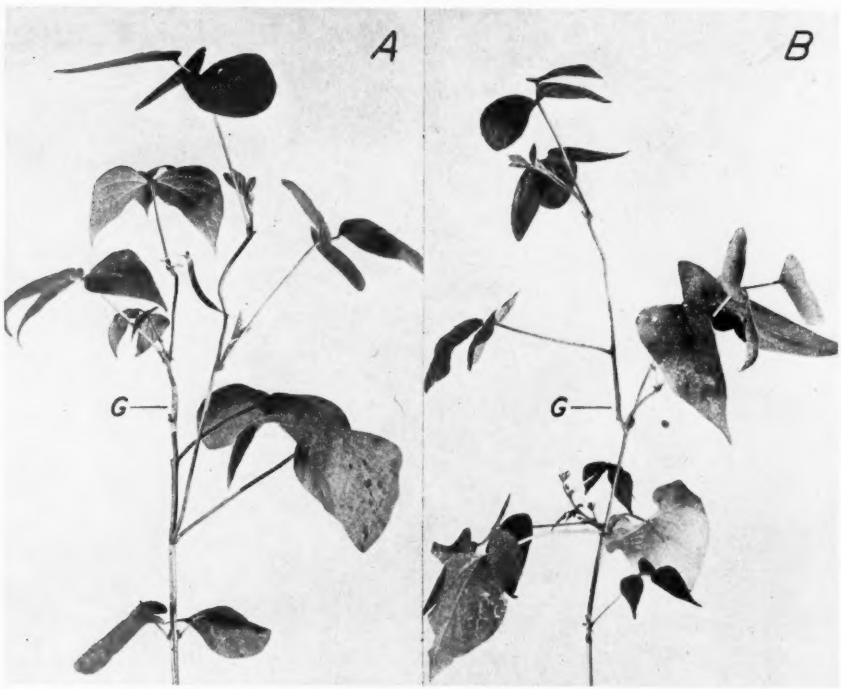

FIG. 3.—Splice grafts of Biloxi soybean and Red Kidney garden bean stems. *A*, Red Kidney scion on Biloxi stock. *B*, Biloxi scion on Red Kidney stock (*G*, graft union). Photographed 6 weeks after grafting.

plants were placed in a saturated atmosphere, where they were kept for 6 or 8 days. They were then returned to normal greenhouse conditions. At all times the plants continued to receive long photoperiods.

The plants used as stocks for the stem grafts had started to expand their second compound leaf at the time the grafts were made. Those from which the scions were taken varied in stage and type of development from one experiment to another. The scions, always $1\frac{1}{2}$–2 inches long, included a partially expanded leaf and the terminal bud. The stems of the stocks were severed above the first com-

pound leaf. Slanting cuts were made through both stock and scion and the two surfaces brought together and securely wrapped with Parafilm (figs. 2*C*, 3). All stem grafts were given the same moist-chamber treatment as the leaf grafts.

A few bud grafts were made by grafting a bud of one plant on to the node of another plant where a bud had been removed. Enough extra tissue above and below the grafted bud was retained to permit wrapping without covering the bud. The top of the plant was removed in order to force the grafted bud. The plants bearing the bud grafts were placed in the moist chamber for 10 days, then removed to the greenhouse bench.

The presence or absence of flower primordia in buds of the receptors was determined in all the experiments by dissection 2–3 weeks after grafting.

Results

Grafts of several varieties of Soja max to Biloxi variety

Approach grafts.—In the spring of 1941, two preliminary experiments were conducted involving approach grafts of Agate to Biloxi soybeans. In experiment I, forty-five grafts were made; and 18 days after grafting, flower primordia were present on eleven of the receptors. In the second experiment, forty more approach grafts of the same varieties were made, and of these, nine receptors formed flower primordia in the terminal bud. The leaves were then removed from the receptors of sixteen of the thirty-one grafts that failed to form flower primordia in experiment II. All thirty-one grafts were allowed to continue development. Although the terminals of the receptors were destroyed at the time of dissection, lateral buds developed and these were dissected 2 weeks later. Flower primordia were present on twelve of the sixteen defoliated receptors at this time, while the fifteen undefoliated receptors were still vegetative.

These preliminary experiments suggested that a much higher percentage of the Biloxi plants might have formed flower primordia if the receptors had been defoliated. Experiment III was performed to test further the significance of defoliation of the receptor, and at the same time to determine whether age of the plants at the time of grafting was influential. For this experiment, 280 pairs of Agate and Biloxi plants were grafted. Twenty grafts were made on each of 14 days, beginning when the plants were 22 days old and ending when they were 43 days old. At the time of grafting, ten plants of each lot of twenty were defoliated to one leaflet.

In 120 of the 280 grafts, or in about 43 per cent of the total, flower primordia were formed on the receptor plants; the other 160 receptors remained vegetative. Age of the plants at time of grafting did not influence the results. There were minor fluctuations in the numbers of receptor plants bearing flower primordia in the groups grafted on various days, but the results showed no correlation with age.

Defoliation did not result in marked differences in number of plants producing flower primordia. Of the 120 plants that formed flower primordia, sixty-two had been defoliated at the time of grafting and fifty-eight had not been. This result is not in agreement with that obtained in experiment II, but the plants of experiment II were defoliated many days after they were grafted, whereas those of experiment III were defoliated at the time the grafts were made.

In a fourth experiment, five lots of twenty-five Biloxi-Agate approach grafts were made. One lot was not defoliated, another was defoliated at the time of grafting, and the remaining three lots were defoliated 4, 8, and 12 days, respectively, after grafting. In the undefoliated controls nine of the twenty-five receptors formed flower primordia. In the plants defoliated at the time of grafting, twelve of the twenty-five receptors formed flower primordia. In those lots defoliated 4, 8, and 12 days after grafting, seventeen, fifteen, and eighteen plants, respectively, formed flower primordia. Defoliation of the receptor, performed 4 or more days after the plants were grafted, resulted in the formation of flower primordia on more receptors than defoliation performed at the time of grafting.

The failure in all experiments of certain of the receptors to initiate flower primordia, even when they were defoliated several days after grafting, indicated that other inhibiting factors were operating. Although all the grafted plants seemed to have made good unions, there was a possibility that this was not the case. Consequently the roots of all the receptor plants were removed from the soil and allowed to dry, thereby making the survival of the receptors dependent upon the quality of the graft union (fig. 1). Thirty-five days later, only fourteen of the seventy-one flowering plants were dead, but twenty-seven of the fifty-four nonflowering ones were dead. This same trend was observed in another experiment involving forty-five grafts similarly treated. Three weeks after the roots were pulled up, fourteen of these plants were dead. These included 44 per cent of the plants that did not have flower primordia and only 24 per cent of those that had flower primordia. This correlation between high percentage of flower-bearing receptors and low percentage of fatalities among them indicates that the quality of the graft union has some influence on the transmission of a flower-forming stimulus from Agate to Biloxi.

It seems evident from the preceding experiments that in a considerable percentage of cases a flower-forming stimulus from an Agate soybean may cross an approach graft union and result in floral initiation in a Biloxi stock. It would therefore appear possible that, if two Biloxi plants were grafted together, a flower-forming stimulus induced in one by placing it on short photoperiods would move across the graft union and cause floral initiation in the other. Several experiments of this type, involving a total of eighty approach grafts in which both donor and

receptor were of the Biloxi variety, were performed. Forty of the donors were given induction treatment with short photoperiods prior to grafting and forty of them after the grafts were made. Of the donors that were induced prior to grafting, thirty were given four short photoperiods and ten were given seven short photoperiods. Of those induced after grafting, ten were given short photoperiods for 8 days and thirty for 14 days. The receptors in these experiments were treated in three ways: some were not defoliated, others were defoliated to one leaflet at the time of grafting, and still others were defoliated several days after grafting. In all these grafts, only one receptor initiated flower primordia. This plant had been undefoliated and its donor had received eight short photoperiods after grafting.

PETIOLE SPLICE GRAFTS, STEM SPLICE GRAFTS, AND BUD GRAFTS.—In two experiments performed at different times during the fall of 1941, fifty-five Agate leaves were grafted to separate Biloxi receptors (fig. 2). Fifty-three of these grafted leaves survived, enlarged to about their customary size, and remained on the plants as long as ungrafted leaves of similar age. Flower buds were initiated on all fifty-three receptors but were not formed on the other two plants on which the Agate leaf failed to survive. As controls for these experiments, noninduced Biloxi leaves were grafted to Biloxi receptors. At the same time Biloxi leaves that had been induced 7 and 12 days were grafted to Biloxi receptors of two other lots, respectively. Floral initiation occurred on none of the receptors to which either induced or noninduced Biloxi leaves were grafted.

At the time of dissection, 2 weeks after grafting, there were many more flower buds on all the Biloxi receptors to which a single Agate leaf had been splice grafted than were present on the receptors to which an entire Agate plant had been approach grafted. The flower primordia on receptors of approach-grafted plants usually occurred at only a few nodes, and the younger buds that differentiated above these were vegetative in every case. On the receptors to which Agate leaves were splice grafted, all the buds differentiated after the first flower primordia were formed became flower buds.

Since abundant flower buds were present on Biloxi receptors 2 weeks after Agate leaves were grafted to them, the stimulus causing their initiation must have reached the meristems of the receptors very quickly. An indication of the length of time necessary for an Agate leaf to remain grafted to a receptor plant in order to result in floral initiation was obtained by removing the Agate leaf from the receptor at various times after grafting. For the first experiment of this type an Agate leaf was grafted to each of the thirty Biloxi plants. After 1, 2, 3, 4, 6, and 8 days, the grafted leaf was removed from lots of five receptors. At the time of dissection, flower primordia were present on three, four, and five of the

receptor plants on which an Agate leaf had remained for 4, 6, and 8 days, respectively, but were absent on receptors from which the Agate leaf had been removed after 1, 2, or 3 days.

In another experiment, also with thirty grafted Biloxi plants, the Agate leaves were removed from ten of the receptors after 3, 4, and 6 days, respectively. No flower primordia were formed on the ten receptors of the 3-day lot, but they were present on four and six receptors of the 4- and 6-day lots, respectively.

Batorawka soybean leaves are as effective as Agate leaves in producing floral initiation on Biloxi receptors. A Batorawka leaf was grafted to each of thirty receptors, and on all the receptors flower primordia were initiated.

Flower-bud initiation occurs on Biloxi receptors following splice grafting of stems as readily as following splice grafting of leaves. Twenty-eight splice grafts of stems of Biloxi and Agate were made. In eighteen of these the Biloxi receptors were the scions and in ten they were the stocks. Flower primordia were formed on the receptor components of all twenty-eight grafts. Likewise, in twenty Biloxi-Batorawka stem splice grafts, flower primordia were formed on all Biloxi receptors. In ten of the grafts the receptor was the scion and in the others it was the stock. No flower primordia were formed in the terminal buds of ten Biloxi-Biloxi stem grafts in which both stock and scion were grown continuously on long photoperiod.

A few Biloxi buds were grafted to Agate plants. About half the grafted buds survived. These developed into short branches, in the buds of which flower primordia were formed.

Grafts of several varieties of Phaseolus vulgaris to Biloxi soybean

A flower-forming stimulus passes readily across certain types of graft unions between Agate or Batorawka soybean and Biloxi and results in the initiation of flower primordia on the Biloxi receptors. Grafting experiments were conducted to determine whether or not a similar stimulus originating in plants of *Phaseolus vulgaris* might cross a graft union to a Biloxi plant and cause flowering. The varieties of garden beans used were Plentiful, Black Valentine, Red Kidney, and Dwarf Horticulture. Flowering in these varieties is influenced very little by length of photoperiod.

The earliest stages of flower primordia are initiated on many varieties of garden beans soon after the seedlings emerge from the soil. Grafts of Biloxi soybeans with garden beans are almost invariably successful, and the scion—whether Biloxi soybean on Red Kidney bean or Red Kidney on Biloxi soybean—makes vigorous growth (fig. 3). Likewise, petiole splice grafts of garden bean leaves to Biloxi soybean plants nearly always succeed. No flower primordia, however, were formed

on any of the sixty Biloxi receptors to which *Phaseolus* leaves were grafted, or on any of the thirty Biloxi receptors to which *Phaseolus* stems were grafted.

Since the scions (in the case of the stem splice grafts) and the leaves (in the case of the petiole splice grafts) become firmly attached, maintain their turgidity, and make further growth after the grafts are made, it seems evident that the translocation of water and probably of mineral nutrients occurs across the graft union. There is evidence, however, that elaborated food materials move across the graft union with difficulty or not at all. For example, a Red Kidney bean leaf that has been grafted to a Biloxi soybean for a week or more does not lose its accumulated starch when left in the dark for many days. Control Red Kidney leaves growing on Red Kidney plants lost most of their starch content the first night. Moreover, an abscission layer eventually develops across the base of the Biloxi petiole to which such leaves are grafted. The stump of the Biloxi petiole turns yellow while the petiole of the *Phaseolus* leaf remains green. The loss of color and abscission of the Biloxi stump occur at about the same time as in similar stumps of petioles to which no *Phaseolus* leaf is grafted. This is in contrast to the behavior of Agate or Batorawka leaves splice grafted to Biloxi. Such leaves remain attached to the Biloxi plant many weeks longer than *Phaseolus* leaves similarly grafted.

Root formation on *Phaseolus* scions constitutes further evidence of lack of food transfer across Biloxi-*Phaseolus* graft unions. These roots form at the base of stem scions and at the lower end of the *Phaseolus* petiole in the petiole splice grafts.

Finally, continued development of *Phaseolus* scions on Biloxi stocks or of Biloxi scions on *Phaseolus* stocks depends upon the presence of leaves on the scion. If all of the leaves are removed from the scion, whether it is Biloxi or *Phaseolus*, the scion fails to develop new leaves. But if a small portion of only one leaflet is left on the scion, the scion continues to grow and develop new leaves.

Discussion

The flower-inducing stimulus of a single Agate leaf grafted to an entire Biloxi plant was sufficient to cause flower-bud initiation in all cases. The flower-inducing stimulus in the two varieties must therefore be the same. In Biloxi the formation of the stimulus does not occur unless the photoperiod is less than 14 hours, while in Agate it is formed even under continuous light.

When Biloxi and Agate plants were approach grafted, however, flower-bud initiation resulted in less than 50 per cent of the Biloxi plants in most experiments. Every Agate plant used for the approach grafts had several leaves, each of which was capable of supplying a flower-inducing stimulus. A more than adequate stimulus, therefore, must have been produced by the Agate plants. Failure of the re-

ceptors to form flower primordia evidently resulted from inadequate transfer of this stimulus to them. Defoliation of the receptors several days after grafting increased the percentage of those initiating flower primordia. Evidently defoliation aided the transfer of the stimulus from the Agate to the Biloxi plant. Such treatment decreased the amount of organic substances synthesized in the defoliated plants and presumably changed the translocation gradient in such a way that an increase in net movement of materials from the Agate to the Biloxi component may have resulted.

Similar results from defoliation have been obtained in other photoperiodic experiments (1), in which two-branched Biloxi soybean plants were used. In these experiments one branch received several short photoperiods while the other was subjected continuously to long photoperiods. Floral initiation occurred abundantly on the short-day branches but occurred only on those long-day branches from which the leaves had been removed at the time the induction treatments were given. The results of this experiment could likewise be explained on the basis of a modified translocation gradient. On the other hand, it could be assumed that the effects of the flower-inducing stimulus of the donor leaves may have been nullified by some inhibiting substance produced by the leaves of the receptors which were on long photoperiods, or that the stimulus from the donor leaves was absorbed by the leaves of the receptors to such an extent that insufficient amounts reached the growing point with sufficient intensity to cause floral initiation—in spite of the fact that all but one of the Biloxi leaves were still present on the plant.

Biloxi leaves that had been subjected to several short days before they were grafted failed to induce the formation of flower primordia on receptor plants. If flower primordia had been formed on the receptors this would have meant that the grafted leaf had undergone some change resulting from the induction treatment that enabled it to continue liberating a flower-inducing stimulus after it had been returned to long photoperiods. Failure of the grafted leaves to induce flower primordia on the receptors is in agreement with the results previously obtained in which it has been shown that the flower-inducing stimulus does not continue after the plants are returned to long photoperiods.

Although all donors produced abundant flower buds in the Biloxi-Biloxi approach grafts, only one receptor formed flower primordia. The flower-inducing stimulus was evidently present in the donors but for some reason failed to reach the terminal meristems of the receptors in sufficient amount to result in flower-bud formation. The reasons for this are not known.

It has been shown previously (3) that the stimulus resulting in floral initiation in the Biloxi soybean will readily move either up or down the plant. Further evidence that this is true was obtained from the stem splice-grafting experiments between Agate and Biloxi. Since flower buds formed on the receptors, whether they

were scions or stocks, it is evident that the flower-inducing stimulus crossed the graft union in either direction. KUIJPER and SCHUURMAN (8) did not observe an upward translocation of the stimulus in the soybean varieties they grafted.

Summary

1. Biloxi plants or parts of Biloxi plants, subjected continuously to daily photoperiods of 17 or more hours, were used as one component of all grafts reported in this work. Plants of this variety do not develop sufficient flower-forming stimulus on long photoperiods to cause floral initiation. They therefore served to determine whether or not a flower-inducing stimulus was transmitted across the graft unions. They are referred to as receptors and the plant or plant parts grafted to them as the donors.

2. The donor components were from Agate, Batorawka, or Biloxi varieties of *Soja max*, or from Red Kidney, Plentiful, Black Valentine, or Dwarf Horticulture varieties of *Phaseolus vulgaris*.

3. Methods of grafting employed were approach grafting of stems, splice grafting of petioles, splice grafting of stems, and bud grafting.

4. A total of 490 Agate-Biloxi approach grafts were made and all formed strong unions. Approximately 50 per cent of the Biloxi receptors formed flower primordia. This percentage was somewhat greater if the receptors were defoliated a few days after they were grafted.

5. Eighty Biloxi-Biloxi approach grafts were made. Flower primordia were formed on no receptors whose donors received long photoperiods continuously, and on only one receptor whose donor received short photoperiods after it was grafted.

6. Eighty-five Agate or Batorawka leaves were grafted to Biloxi receptors. Flower primordia developed on all eighty-three receptors on which the grafted leaf survived.

7. Formation of flower primordia on Biloxi receptor plants to which an Agate leaf was grafted occurred only when the grafted leaf remained on the receptor 4 or more days.

8. Flower primordia developed on all the Biloxi receptors of the forty-eight Agate or Batorawka stem grafts made. The flower-inducing stimulus passed either up or down through the graft union, depending upon whether the receptor was the scion or the stock.

9. Thirty grafts of Biloxi leaves on Biloxi receptors, and twenty of Biloxi stems to Biloxi receptors, were made; all lived, but flower primordia were formed on no Biloxi receptors. In some of these grafts the Biloxi donors had received long photoperiods at all times; in others they had received various numbers of short photoperiods prior to grafting.

10. Sixty leaf grafts and thirty stem grafts of *Phaseolus* bean to Biloxi soybean receptors were made. Of these, fifty-nine of the former and twenty-seven of the latter survived, but flower primordia were formed on no Biloxi receptors.

11. Water and probably mineral nutrients move across *Phaseolus*-Biloxi graft unions readily, but elaborated foods do not appear to cross such unions.

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GROWTH AND DEVELOPMENT IN RANGE GRASSES. II. EARLY DEVELOPMENT OF BOUTELOUA CURTIPENDULA AS AFFECTED BY DROUGHT PERIODS

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Introduction

Studies on the development of *Bouteloua curtipendula* as affected by watering frequency have been reported previously (**9**). In conjunction with that research it seemed desirable to determine the effects of severe soil drought upon seedlings watered at various frequencies, at different developmental stages. Both soil and atmospheric drought periods may be considered part of the normal environment during seedling establishment of most range grasses. In cereals, the occurrence of critical periods in development, during which limited water supply has greatest effect, has long been known (**1, 7**). In certain spring wheats, AAMODT and JOHNSTON have shown that superior drought resistance is associated with (*a*) ability to evade early periods of drought; (*b*) rapid development of primary root systems when young, both in branching and number of primary roots; and (*c*) superior capacity to endure drought without permanent injury. In perennials, equally important must be the ability of certain parts, usually underground or nearly so, to remain dormant but alive during periods of soil drought which cause death or dying back of tops (**2**).

Few workers on range grasses, even when recording death of parts (**11**), have considered carefully the morphological pattern of individual species in relation to the problems of drought resistance or the possible effects of drought upon this pattern. In general, only those anatomical modifications considered to be xeromorphic, or the rate and depth of root penetration and root branching, have been considered. The differential availability of a limited water supply to different organs within a plant, in conjunction with differential protection from—and resistance to—desiccation, may lead to interesting developmental patterns. In maize (**6**) it has been suggested that stunting effects tend to be most serious during the formative period; that is, during the first 30–40 days of the plant's life, when the developmental pattern is laid down. In certain fruit trees, drought conditions during the period of floral differentiation may result in excessive flower-bud formation (**4**), while in cotton the flower buds are shed (**5**). GLENDENING (**3**) reported flowering by *Heteropogon contortus* in 7–36 days following resumption of watering after the subjection of 7-weeks-old plants to a drought period of 6 weeks. Plants, grown at medium levels of water supply preceding this period, flowered earlier

than those first subjected to dry or wet conditions. He gave no data for plants not subjected to drought.

In addition to gross observations on drought effects, including death of parts, some attempt was made in the present study to follow the rate and degree of development of individual organs initiated just before, or early in, a drought period, together with any correlative effects produced in the plant as a whole when watering was resumed. As previously indicated, *Bouteloua curtipendula* was chosen for study because of its considerable increase in importance in various parts of the central grassland region during the early years of the drought period. A study of its early development in relation to water supply and drought might aid in interpreting this increase.

Methods and environmental conditions

Procedure and general growth conditions during the experiment, except soil moisture, have been reported (**9**). Plants were grown from seed in 2-gallon glazed crocks, and were thinned to ten plants per crock. Water was supplied to three basic series in an amount equivalent to 0.4 surface-inch at intervals of 3, 6, and 12 days. From these series, four or five crocks of each were set aside to dry when plants were 18, 30, and 42 days old. In a fourth series, watered every 20 days, five pots each were placed on drought after 26 and 46 days. In all cases, the beginning of drought periods coincided with the end of a normal watering interval, with the measurement of soil moisture, and with root and top development in the four basic series (**9**, tables 1, 3–6), the latter series having been set up on November 27, when the plants were 6 days old. All series were given the regular watering of 0.4 surface-inch on the first day of their drought periods. This produced the wettest condition at the beginning of the dry period in the series previously watered most frequently (at intervals of 3 days), while driest conditions were found in those first watered at 20-day intervals. In the latter, both at 26 and 46 days, soil-water content had been reduced almost to the permanent wilting percentage. Among pots with a 3- or 6-day watering interval preceding drought, those placed on drought at 42 days were also much wetter than those allowed to dry earlier. These conditions were not disadvantageous, because the larger plants in both the older and wetter series reduced the water content during drought much more rapidly than did the smaller plants which began drought under seemingly much drier conditions. The net result was probably to impose more nearly the same intensity of drought on all series than could have been done in any other way under the experimental conditions. After 48 days of drought treatment, soil-moisture content in all series and ages was reduced below the permanent wilting percentage, and ranged from 0.1 to 0.5 per cent above the hygroscopic coefficient of 0.8 per cent. As judged by soil-water content, duration and degree of wilting,

death of foliage, and length of time required for recovery, water deficit became somewhat more acute in all series placed on drought when 42 days old than in the others, and was least severe in those where drying started at 18 days.

After 48 days of drought, plants from one crock in each series were washed out for measurement. One pot of each was left dry to complete total drought periods of 88, 80, 76, 69, and 65 days for series allowed to dry when 18, 26, 30, 42, and 46 days old, respectively. These latter pots were then watered amply to test survival and amount of recovery during the following week. The remaining three pots in each series were given 0.4 inch of water after 48 days of drought, and one pot of each was washed out after 3 days for a record of renewed growth. The remaining two pots were watered (0.4 inch) at intervals of 3 or 12 days until the close of the experiment, to allow recovery under both wet and dry conditions. All remaining plants were harvested at 119 days. Hereafter in referring to treatments, numerals such as 3–18–48–3 will indicate, in order, plants watered at intervals of 3 days until 18 days old, followed by a drought treatment of 48 days, after which watering was resumed at 3-day intervals until 119 days old. Total amounts of water supplied to series left on drought for 48 days ranged from 9.6 surface-inches in 3–18–48–3, 3–30–48–3, and 3–42–48–3 to 2.8 in 20–26–48–12 and 20–46–48–12. Several other treatments received the same total amounts of water, including 12–18–48–12, 12–30–48–12, and 12–42–48–12 (3.2 inches). Harvesting and measurements of growth and development followed the procedure outlined in the previous report (**9**)

Results

Primary root system

The imposition of a drought period, if not too severe, probably tended to prolong the life of the primary root system. It was still alive in the great majority of the 220 plants, examined at 119 days, in the various treatments involving 48 days of drought. This is in contrast to its relatively early death in the continuously wet series, previously reported (**9**). It was found dead most frequently in those series, such as the 3–42's or 6–42's, in which frequent watering and greater age had allowed establishment of a number of adventitious roots from the primary axis before the drought period. It was the only functional absorbing organ in thirty-three plants, all alive, of the 220, and in fourteen others was supplemented only by one to four roots developed from secondary axes after the resumption of watering at 3-day intervals. Of twelve plants, however, which either had died by the end of the 48-day drought or had failed to recover on a 12-day watering interval, nine had not established adventitious roots, suggesting the inadequacy of the primary root system alone during severe stress in some plants. In three of the nine, death actually resulted from breaking of the mesocotyl. The forty-two plants, either living or dead, which had failed to establish adventitious roots were found in

fourteen of the twenty-two treatments, but only two of these had been watered at 3-day intervals preceding drought.

In those pots subjected to drought for periods longer than 48 days, only fifteen plants out of 107 were alive when harvested. They were found in four pots—3–18–88, 3–30–76, 6–30–76, and 3–42–69. Of the thirty-eight plants in these four pots, fourteen of the fifteen alive had established one or more adventitious roots. Of the thirty-nine plants placed on drought when 18 or 26 days old, five of the six still alive at 119 days showed establishment of the first adventitious roots to be formed, while only the primary root had become functional in the thirty-three which had died. Of the thirty placed on drought at 30 days, the eight remaining alive had a range of one to five established adventitious roots in contrast to none to three in the dead plants. These data illustrate the importance of early establishment of adventitious roots in seedling survival of severe drought.

The influence of drought periods on degree of branching and penetration of the primary root system was impossible to determine, since these roots are so slender as to undergo considerable breakage in removal from soil. It was observed generally, on plants washed out immediately at the close of drought periods, that differentiation of root tips in soil had apparently occurred to within a fraction of a millimeter from the tip, in both primary and secondary root systems, as judged by root-hair production and other criteria. These tips renewed growth very slowly from the residual meristem and in most cases had elongated only 2 mm. or less on plants washed out 3 days after watering was resumed. In some cases no new growth was apparent, even on tips which still seemed alive.

Primary stem, leaves, and tillers

Death of primary leaves and of those on secondary axes proceeded from the tips downward, from the outer to the inner margin of the convolutely-rolled wilted leaves, and from lower to higher positions on each axis, until death of stem tissues occurred. There were no consistent differences in percentage of dead foliage among the different treatments. In practically all plants subjected to drought for 48 days, almost all foliage had died which was not protected by a favorable position in vernation upon wilting and rolling. Generally only the one or two leaf primordia which had not yet partially emerged from the tube of leaf bases, and the basal portions of sheaths or inner halves of the convolutely-rolled blades of the next two or three older leaves, remained alive on the various axes. Protection afforded the growing points seemed fairly effective, because in only a few of the plants alive after the 48-day drought had any primary or secondary stem tips died. In these few plants, in those remaining alive after longer drought periods, and in those which failed to recover, death occurred first in stem meristems in the most exposed positions above the ground. This condition was often associated with the earlier

death of segments of elongated internodes no longer protected by leaf sheaths. In cereals, the period of "shooting" is regarded as one in which water deficit is most critical (**1, 7**), often leading to death of the primary axis, with consequent delay in heading and reduction of yield after recovery by development of lateral axes.

After the resumption of watering, survival became apparent within 24 hours in most plants by the unrolling of the living portions of leaves and the renewal of growth by leaf and stem meristems, resulting in the rapid emergence of fresh green tissue through the mass of surrounding dead leaves.

The pattern of top growth was not changed markedly by drought, in correlation either with age at time of drought or with previous watering interval, except in those few plants in which stem meristems had died. There was some suggestion of the maturation of one or two more basal unelongated internodes, on both primary and secondary axes, in those plants in which shooting would normally have occurred during the drought period than in the control series previously described (**9**). In other cases, as in 3–30–48–3, there was marked reduction in length of the second or third elongating internode, which differentiated and elongated during the drought period, compared with its length in plants not subjected to drought. Such stunting apparently was not deleterious in further growth of the same axis.

In some individual plants in which rapid differentiation and growth of the first or second primary tillers should have occurred during the drought period, a suggestion of stunting was noted. A larger proportion of first primary tillers had remained small, and there was more of a tendency for the third tiller to be largest than in the series continuously watered. Based on averages, however, the second tiller was taller than the first and third in all treatments at 119 days.

In analyzing the numerous criteria of growth and differentiation of the tops, the twenty-two treatments with a drought period of 48 days were arranged in sequence of total water supplied, together with the four series not subjected to drought. The relative rank of the different treatments, based on living plant averages for numerous growth measurements in the 119-day plants, was likewise determined. Any striking deviations in the latter from the sequence based on total water supplied were easily seen. In general, it was found that drought at different ages, and in conjunction with previous and subsequent divergence in watering frequency, produced no outstanding deviations in rank based on different growth data from that based on total water supplied. Criteria of size, such as height of plant or tillers, or length and number of elongated internodes, usually showed a wider range of values than did those of organ differentiation, such as numbers of primary leaves, tillers, etc. This suggests a greater relative effect of drought on enlargement than on organ differentiation, if meristems remained alive.

Average height at 119 days, in plants subjected to 48 days of drought, ranged from 50 cm. in 6–18–48–3 (8.8 inches of water) to 22 cm. in 20–26–48–12 (2.8

inches). Plants with the same total water supply were approximately equal in height; for example, 3–18–48–3, 3–30–48–3, and 3–42–48–3 (9.6 inches of water) averaged 45, 46, and 44 cm. tall, respectively. Plants 12–18–48–12, 12–30–48–12, and 12–42–48–12 (3.2 inches) averaged 30, 29, and 29 cm. Numbers of primary leaves in the same series were 10.0, 10.2, 9.9, and 8.2, 8.1, and 7.2, respectively. Leaf number ranged from 10.6 to 7.2 in 12–18–48–3 and 12–42–48–12. Average number of elongated internodes on the primary axis ranged from 3.8 in 6–18–48–3 to 0.2 in 20–46–48–12, while in the two groups just listed the averages were 3.7, 3.3, 3.3, and 1.7, 1.3, 0.6.

Average length of the second primary tiller ranged from 36 cm. in 3–18–48–3 to 10 cm. in 20–26–48–12, while leaf numbers were 9.9 in 12–18–48–3 and 6.8 in 20–46–48–12. Heights in the two groups with equal water supply were 36, 30, 27, and 13, 14, 13 cm., while leaf numbers were 9.2, 9.2, 8.9, and 7.6, 7.7, 7.0.

Highest values for (*a*) number of primary tillers over 15 cm. long, (*b*) number of primary tillers showing internodal elongation, (*c*) height and (*d*) number of leaves of largest primary tillers, (*e*) number of secondary tillers and (*f*) those over 15 cm. long, and (*g*) tertiary tillers were 5.2, 2.2, 41 cm., 9.9, 25.7, 5.3, and 21.4. Lowest values for the same criteria were 2.7, 0, 12 cm., 6.6, 12.6, 0, and 0, respectively.

In general, values in the different treatments for all growth measurements fell into or near the sequence based on total water supply. Treatments with most consistent departure from this sequence were 3–42–48–3 and 3–42–48–12, showing lower rank in most growth criteria, and 6–18–48–3, 12–30–48–3, 6–42–48–3, 12–42–48–3, and 20–26–48–12, with higher rank in growth than in sequence of water furnished. In the first two, the lower values may be attributed to inability to use efficiently the abundant water supplied before drought, to a somewhat greater retardation in growth during the drought period owing to the relatively early exhaustion of the water reserve by these largest plants, and possibly to an actual lowered physiological drought resistance owing to the early abundant supply of water. In the other five treatments, some one or all of these three factors may have operated in the opposite direction to allow the more effective development.

Secondary root system

The plan of initiation of adventitious roots on the primary axis has been described in detail (**9**). Numbers initiated near lower nodes during the previously reported study tended to be correlated with frequency of watering. Near the upper nodes, and on secondary axes, any such tendency was counteracted in part by a tendency toward stimulation of primordium initiation when older primordia failed in establishment. In the drought series, both of these tendencies naturally became effective, the drought conditions tending to inhibit initiation as well as establishment, while the failure of the latter during drought apparently stimu-

lated production of new primordia when water again became available. Rate of new production in individual plants often seemed to be inversely proportional to the number previously established, although regulated also by the adequacy of water supply following drought, which controlled further shoot development.

In the great majority of all treatments with 48 days of drought, major deviations in rank at 119 days—from that based on water supply—were found in practically all measurements of root development. The data included averages for: total numbers of primordia initiated on stems; percentage remaining alive; numbers produced on primary and secondary axes and near each node of the primary axis; total numbers of established roots (living roots over 50 mm. long) and totals on main axis and primary tillers; and numbers and percentage of established roots near each node of the primary axis.

Total numbers of root primordia produced showed strong correlation with frequency of watering following drought, those plants with the more frequent watering mostly outranking those watered on 12-day intervals. Values in the former group ranged from 42.7 in 6–18–48–3 to 28.7 in 6–42–48–3; and in the latter, with two exceptions, from 28.3 in 12–18–48–12 to 15.2 in 12–42–48–12. The exceptions, 6–18–48–12 and 20–26–48–12, produced 34.4 and 34.6 primordia, respectively. The latter pot was the only one in which all the ten plants failed to establish any adventitious roots, leading to the most extreme production of primordia in relation to the limited amount of water supplied. In the other, only three of ten plants established one adventitious root each before being placed on drought. In the two larger groups, rank in total primordium production was displaced upward, from that accorded by water supply, in all treatments in which imposition of drought at 18 days, or infrequent watering prior to drought in older plants, had prevented adventitious root establishment. Conversely, rank in primordium production was shifted downward in all treatments favoring early establishment of adventitious roots.

The percentage of primordia and roots, alive at 119 days, ranged from 74 in 6–42–48–3 to 36 in 6–18–48–12. It was obviously influenced both by death of roots from drought and other causes and by the relatively high numbers of living primordia most recently initiated in certain treatments. These two values often compensated each other in the percentage figure.

Total numbers of adventitious roots initiated on the main axis ranged from 14.6 in 12–18–48–3 to 10.1 in 6–42–48–3 and 8.2 in 12–42–48–12. This value was related to a complex of factors: the degree of maturity of all crown nodes and internodes (in some plants, grown under driest conditions, primordia were not yet differentiated near the higher nodes); the tendency of both a drought period and infrequent watering to increase the number of internodes which do not elongate with consequent increase in number of nodes near which primordia are normally

differentiated; and the tendencies for larger numbers to be initiated by plants with a favorable water supply, counteracted by the stimulating effect—both in time and numbers initiated near higher nodes—of previous failure in establishment. Actual numbers of roots initiated on the primary axis could be interpreted only by reference to the differential importance of these factors in the various treatments.

Numbers of root primordia initiated on secondary axes showed approximately the same relation in the different treatments to the sequence based on water supply as did the total numbers per plant. They ranged from 25.4 in 6–18–48–3 to 6.4 in 6–30–48–12.

Live established roots over 50 mm. long on the main axis ranged from 5.2 in 6–42–48–3 to 0 in 20–26–48–12. With the exception of the latter, and 12–42–48–12 (3.6 roots) and 3–42–48–12 (2.9 roots), such roots in all other treatments—with a 12-day watering frequency following drought—ranged from 2.2 to 0.6. In very few of these plants were any adventitious roots established on either primary or secondary axes following drought, emphasizing the need in this species, previously reported, of at least 3 consecutive days of soil-surface wetness for such establishment. Plants watered at 3-day intervals following drought, except 20–46–48–3 (1.8 roots) and 20–26–48–3 (1.1 roots), possessed from 5.2 to 2.6 established roots at 119 days. In this group of treatments one or more roots per plant were established following the resumption of watering. In both groups, the average number of adventitious roots established before drought increased consistently with age at drought in all frequencies of watering, and in general, at each age, with increasing frequency of watering.

Established live roots on tillers were found, with rare exceptions, only on plants watered at 3-day intervals following drought, ranging in number from 5.4 in 3–18–48–3 to 1.1 in 3–42–48–3 and 20–46–48–3. Those earliest subjected to drought consistently had the highest number of established tiller roots in contrast to those allowed to dry when older. This fact is related to the coincidence of the resumption of watering in the former with (or near) the time when first-formed tillers had reached a developmental stage, allowing vigorous root initiation and some successful establishment. In plants placed on drought when older, many primordia, initiated just before or during the early stages of drought, had died before watering was resumed or were too weak to make effective growth in the somewhat arid aerial environment characteristic of the latter phases of the experiment. In practically all plants watered at 12-day intervals following drought, tiller roots failed completely, although initiated in considerable numbers. Death occurred usually because the rate of soil drying exceeded their rates of penetration.

The length of time during which initiated primordia, still buried in stem tissues, retained their capacity for development was shown by analyzing the data with

respect to time of production and establishment of roots near the successive nodes on the primary axis. For example, plants placed on drought at 18 and 26 days had commonly initiated primordia near the coleoptilar (second) node, and in some cases near the third node as well. The former primordia, especially, grew in response to the application of water at this time; and—in those plants previously watered at 3- or 6-day intervals—often became established. Primordia near the third node failed to grow at this time in most treatments, or, having done so, failed in establishment during the beginning of the drought period. During the gradual increase in internal water deficit, primordia were produced successively near the higher nodes, so that at the conclusion of the drought period (plants 66 and 74 days old) roots had been initiated near all nodes up to and including the fifth, and in some cases, the sixth. Upon resumption of watering, however, it was found that only those primordia near the higher nodes, particularly the fifth, were capable of effective growth and successful establishment. The primordia near the third and fourth nodes had often died, still buried in apparently living stem tissue; or, if still alive, seemingly showed cell maturation to the tip and incapacity for growth. In other cases it was obvious that great amounts of mechanical tissue had differentiated from the surrounding stem cells, possibly restricting the activity of the roots.

On plants placed on drought when older, primordia near somewhat higher nodes failed to develop into functional roots, for the reasons already suggested. Thus, plants placed on a 48-day drought at 42 days, after frequent watering, followed by watering at 3-day intervals, usually showed functional established roots near the second and third, and occasionally the fourth, nodes. Most primordia near the fourth and fifth nodes died, either with or without elongation, during the drought period. New roots grew, after watering, from near the sixth, occasionally the fifth, nodes; but so slowly in many cases as to dry before they could become established. In addition to moist soil, a humid atmosphere for at least 3 days was apparently essential to these plants in alleviating internal water deficit sufficiently to permit root establishment.

All adventitious roots, having become established in the soil to a depth of 50 mm., seemed equally capable of survival on all plants remaining alive through the drought period, irrespective of position or age. Their growth was undoubtedly limited by the size of the container, and it is probable that fewer plants with these roots would have died on the longer drought periods if a greater volume of soil had been available for them. In nature, drought survival of many grasses in arid regions must be dependent, during drought dormancy, upon two major factors: first, the meager supplies of water furnished to the basal stem meristems by those roots penetrating into soil layers in which water content is not yet reduced to complete nonavailability; and second, any physiological adaptations, involving bound

water or related phenomena, in the meristems themselves. The zones of possible absorption in such roots might warrant investigation, in view of apparent maturation almost to the tip during drought, reported in this study.

Discussion

In considering the effects of drought on correlations within the plant, as illustrated by its differing effects on initiation and growth of stems and leaves in contrast with roots, the positive correlation in the growth of the former with the amount of water supplied over the 4 months of the experiment seems somewhat remarkable. If considered from the standpoint of averages, it suggests that—aside from those plants which failed to survive the drought period—the differing number, position, and time of establishment of adventitious roots had little effect in producing differential survival and growth of the various parts of the shoot system. Correlations were practically lacking also on an individual plant basis. If progressive determinations of absorbing surface were practical, or if it had been possible to obtain accurate dry-weight data on roots, correlations of these values with the various criteria of shoot growth might have been positive. Even this, however, would not explain the inference that in plants, up to 4 months old, water transmitted from the various adventitious roots—regardless of position—and from the primary root system apparently is equally available to all shoot-growing tips. In individual plants there was some suggestion that any exceptionally vigorous tillers had established adventitious roots, either before the drought period or soon after the resumption of watering. A cause and effect relation might be implied, which undoubtedly becomes operative in older plants.

The bearing of this study upon possible reasons for the early increase of *Bouteloua curtipendula* in the prairie during the years of drought is somewhat perplexing. WEAVER and ALBERTSON (10) attributed this increase partly to the prolific seeding habit of the species, suggesting that seedlings played an important role in the increase. Without causal data on mortality and survival of seedlings of different ages in this region during this period, correlations with the results of this study are impossible. But the latter indicates that such survival and increase can be considered remarkable. Aside from the rapid and deep penetration of its single primary root, this species shows no morphological equipment during its early seedling life which would indicate high drought resistance or success under arid conditions. The lack of more than one seminal root, the production of the first adventitious root when 2 or more weeks old, the slow rate of initiation of additional ones, the period of soil wetness required for their successful establishment, the apparent inability of the first-formed primordia to remain functional through more than 2–3 weeks of internal water deficit, and the establishment of the crown nodes at the surface of the ground in any depth of planting so far used by the

writer—these would all seem to be disadvantageous. Against this may be noted, at least for older plants, the amount of branching and rate of penetration of established adventitious roots, the stimulation in root-primordium production when adventitious roots have failed in establishment, and the apparent equal availability of a limited water supply to all stem growing points located near the ground, together with their protection against desiccation. These growing points are the meristems which eventually give rise to new roots as well as leaves. It may well be that drought resistance in perennial grasses rests partly upon the success of the stem meristems in obtaining their full share of a limited supply of water. In this species they were obviously more efficient in this respect than were those of the roots, which renewed growth very slowly upon resumption of favorable conditions, in contrast with the stem meristems. In this connection, it is well to bear in mind that the pathway and mechanism of transmission of water to stem growing points when drought-dormant grasses resume growth has never been completely determined (**8**).

Summary

1. Seedlings of *Bouteloua curtipendula* in 2-gallon glazed crocks, grown under wet to dry conditions as regulated by watering at intervals of 3, 6, 12, or 20 days, were subjected to soil-drought periods from 48 to 88 days in length. Plants in the first three series were subjected to drought when 18, 30, and 42 days old, and in the last at 26 and 46 days. Watering was resumed in some pots of each series after 48 days of drought, at intervals of 3 and 12 days. Others were left on drought until approximately 110 days old and were then watered amply to test survival. All plants were harvested at 119 days, except those harvested at the beginning, at the close, and 3 days after the close of the 48-day drought.

2. The few seedlings which failed to survive the 48-day drought, and many of the larger number failing to survive for longer periods, had failed in establishment of adventitious roots, either because of having been placed on drought prior to, or just at, the time of initiation of such roots, or because infrequent watering prevented their establishment. Such establishment seemed to be dependent upon 3 consecutive days of soil-surface wetness. A number of small plants, however, survived 48 days of drought with no established adventitious roots.

3. All stem meristems nearest the ground, and the young foliage surrounding them, were equally and most resistant to the effects of internal water deficit, resuming growth rapidly after watering. Older foliage died during the 48-day drought period. Initiated adventitious root primordia, buried in stem tissues, either died or failed to grow effectively after subjection to 2–3 weeks of water deficit. Many root tips, established in the soil, had apparently survived but resumed growth very slowly after drought, in contrast to stem meristems.

4. There was little differential effect of drought at different ages on the growth produced by all stem meristems which survived. At 119 days, numerous growth measurements were correlated closely with total amount of water supplied during the entire experiment. In general, size was reduced by drought to a greater degree than were numbers of organs differentiated, at least in the 48-day period, as compared with continuously watered controls.

5. Drought, when it prevented establishment of adventitious roots, tended to stimulate the initiation of root primordia on the various axes, both by acceleration of rate of initiation and by increase in total numbers produced on an axis, after watering was resumed.

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QUANTITATIVE STUDY OF ETHYLENE PRODUCTION IN RELATION TO RESPIRATION OF PEARS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 538

ELMER HANSEN

(WITH SEVEN FIGURES)

Introduction

Recent investigations have shown that volatile substances other than carbon dioxide are produced by apples (**2, 4**), pears (**8**), and certain other fruits (**15**). These emanations, although present in minute amounts, have been found to influence the respiration and other metabolic activities associated with ripening processes. That the active principle concerned with producing these effects is ethylene has been established definitely (**3, 9, 20**). In view of these findings, the factors associated with the formation of ethylene in fruits and its production in relation to other phases of metabolism have been studied.

GANE (**4**) has measured the quantity of combustible emanations from Marie Louise pears and found that the amounts produced increased rapidly at the climacteric. With apples he found some indication that the more rapidly respiring varieties were in general more active in the production of volatiles. KIDD and coworkers (**12**) have estimated by combustion the total carbon-containing substances other than CO_2 which are evolved during the ripening of Conference pears stored at 10° C. and found that the maximum rate of production of these emanations corresponded to the maximum of the initial rise in respiratory activity. They did not find that this increase was due to an increase in the rates of escape of alcohol and acetaldehyde. GERHARDT and EZELL (**5**), using concentrated sulphuric acid as an absorbent, studied the emanation of volatiles in relation to respiration of Bartlett pears during ripening at 65° F. and found that the climacteric for respiration preceded that for total volatiles by approximately 7 days. They state, however, that a considerable portion of the emanations from fruits of this variety may result from the gross liberation of acetaldehyde from the tissues.

The development of microchemical methods (**1, 16, 20**) for the determination of ethylene has made it possible to isolate ethylene from all other volatile compounds produced by fruits and to estimate it separately. NELSON (**16**), by use of a potassium-permanganate oxidation method, has made a quantitative study of ethylene production by apples and bananas and has shown that an apparent

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relationship exists between the keeping qualities of apples and the amounts of ethylene which they produce.

The present investigation was undertaken for the purpose of making a quantitative study of ethylene production in relation to respiration of pears. The emanation of this gas during ripening in air at 20° C.—and under conditions by which respiration was altered by changes in temperature and oxygen tension—has been studied.

Material and methods

SOURCE AND HANDLING OF FRUIT.—The three varieties of pears—Bartlett, Doyenne du Comice, and Beurre d'Anjou—used in this investigation were obtained from the Medford, Oregon, district. The fruit was picked at the regular commercial picking date for the variety, packed in oil wraps, and stored at 0° C. (32° F.). During the fall of 1939, the fruit was shipped under refrigeration to the University of Chicago, where the experimental work for the first season was carried out. The methods used in handling the fruit after removal from cold storage varied with different lots, as later specified.

ANALYTICAL METHODS.—The method for determining the CO_2 and O_2 of respiration was that of HALLER and ROSE (**7**) as modified by MASURE (**14**). Approximately 2.5 kilograms of fruit were inclosed for 15–20 hours each day in large desiccators. Oxygen was supplied from calibrated 2.5-liter bottles connected to the desiccators by means of 1-mm. capillary tubing. The oxygen inlets were fitted with mercury valves to prevent backward diffusion of air and the evolved ethylene into the oxygen-supply bottle. The fruit was held at 20° C. (69° F.) during ripening, except for certain experiments as later specified. The use of this method (in which the fruit is kept in a confined air space) made it possible to determine CO_2, O_2, and ethylene on the same fruit sample. In this procedure the evolved ethylene accumulates in the desiccators during confinement of the fruit, and this fact must be taken into consideration in interpreting any data obtained. The recent work of KIDD and WEST (**11**) and HULME (**10**), however, has shown that mass lots of fruit cannot be kept free from ethylene, even where ventilated, so that it tends to exert an effect under any method used unless individual fruits are kept isolated from one another.

Ethylene was estimated by the micro-bromination method previously described (**1, 9**). In order to utilize a larger gas sample for analyses than originally specified, the following modified procedure was used. After an aliquot of the potassium-hydroxide solution had been withdrawn for titration, the desiccator was connected to a 220-ml. gas-sampling bulb and a sample of air for ethylene analysis withdrawn. Then 0.2 ml. of 28 per cent ammonium-hydroxide solution was introduced from a pipette connected to the side arm of a three-way stopcock on the lower end of the sampling bulb by means of a short piece of rubber tubing. The

leveling bulb was then raised and the ammonia—together with approximately 10 ml. of mercury—was allowed to mix with the gas sample. The bulb was then shaken and allowed to stand for 15 minutes to insure complete absorption of any acetaldehyde present. A portion of the gas sample was finally passed slowly through the purification unit, as previously described, and collected over mercury in a 100-ml. gas burette.

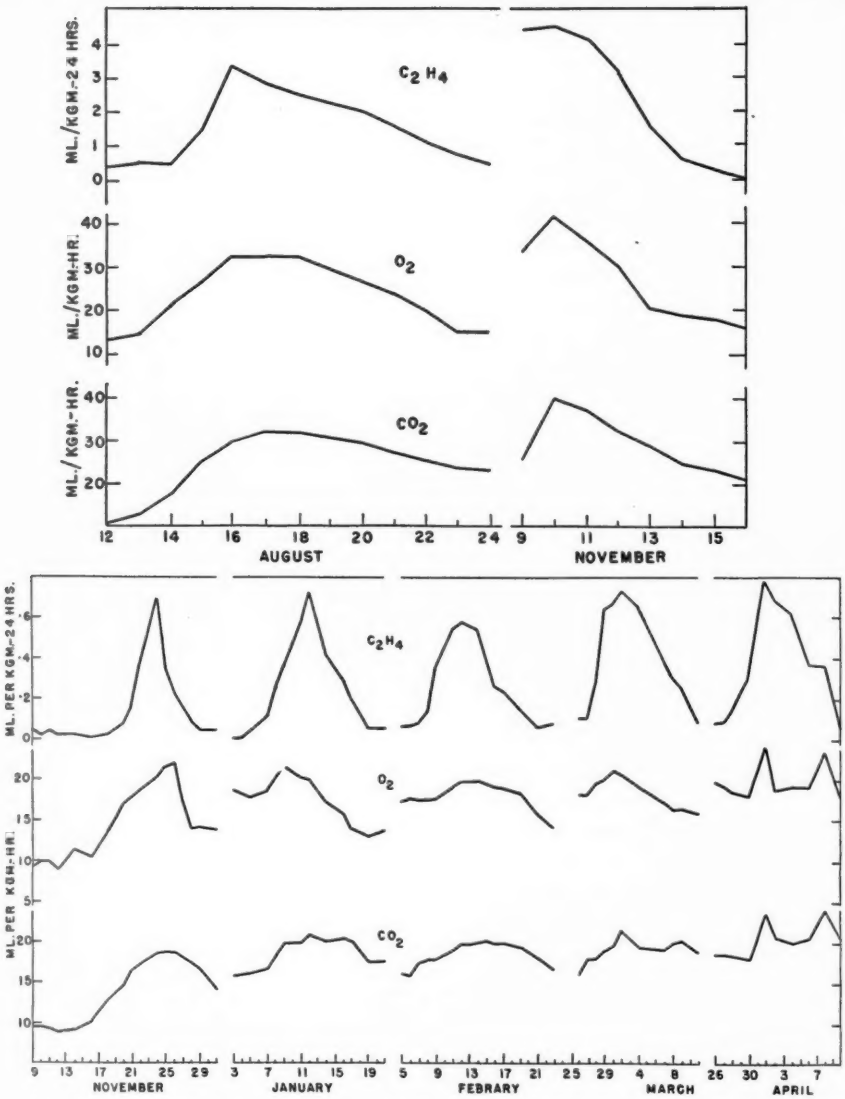
To a 125-ml. reaction flask, identical in design except for size to the one previously described, was added 10 ml. of 0.0025 N potassium bromate and 1 ml. of 6 N sulphuric acid. The flask was evacuated to approximately 27.5 inches of mercury, connected to the burette, and the purified ethylene sample then drawn in by opening the stopcocks and raising the leveling bulb. Since mercury brominates readily, care was taken to prevent its introduction into the flask. To guard against this, a short piece of ordinary tobacco pipe-cleaner was inserted into the capillary outlet of the gas burette. After introducing the gas sample, 2 ml. of N/10 potassium bromide was added from a pipette connected by rubber tubing to the reaction flask. The flask was then shaken for 15 minutes on a horizontal mechanical shaker operating at approximately 100 oscillations per minute. Two ml. of 2.5 per cent potassium iodide was then introduced. After vigorously shaking the solution, a wash bottle was connected to the flask and the neck washed down by drawing in water by means of the residual vacuum. To insure complete absorption of the free bromine, the flask was allowed to stand for 2 minutes, with occasional gentle rotation. The stopcock was then removed, rinsed down carefully with distilled water, and the solution titrated with 0.0025 N sodium thiosulphate from a 10 × 0.02 micro-burette. Duplicate determinations were made on each gas sample.

Experimental results

Relation of ethylene production to respiration during ripening at 20° C. before and after storage at 0° C.

In order to follow the trend of ethylene production in relation to respiration during ripening, samples of fruit were withdrawn at different intervals throughout the storage period and ripened at 20° C. Two varieties of pears were used for these studies. One lot of Bartletts was ripened immediately after picking and the second after being held in storage for 3 months. Five lots of Anjous were withdrawn at approximately monthly intervals, beginning on the third month. The CO_2 and O_2 of respiration, as well as the amounts of ethylene evolved, were determined daily during ripening.

The Bartlett pears ripened immediately after picking showed a low rate of respiration as well as emanation of ethylene at the beginning of the experiment (fig. 1). Both processes began to increase at the same time, but the peak in ethylene production was reached one day earlier than the maximum for respiration.



FIGS. 1, 2.—Fig. 1 (top), ethylene production and respiration of Bartlett pears during ripening before and after storage at 0° C. Fig. 2 (bottom), ethylene production and respiration of Anjou pears during ripening after progressive storage periods.

During the post-climacteric period, ethylene production and oxygen consumption fell off rapidly, while the production of CO_2 showed a much slower rate of decline. The ratio CO_2/O_2 changed markedly during the course of ripening. At the beginning the value was approximately 0.8. At the climacteric, when the fruit was fully ripe, it had increased to unity but rose to 1.4 at the termination of the experiment. These wide changes in the respiratory quotient indicate that qualitative as well as quantitative changes occur in respiration during ripening.

The sample of Bartlett pears held for 3 months in cold storage was still fairly firm but had begun to show a yellow color when withdrawn for ripening. The fruit became fully ripe after being held for 4 days at 20° C. The curves for respiration and ethylene production for this lot differ in some respects from those of the fruit ripened immediately after picking. The peak in respiration was reached on the second day as compared with the fifth day for the first lot. Ethylene production was at a maximum on the second day and declined steadily thereafter, finally falling below the amount that could be quantitatively measured.

The data for the Anjou pears are presented in figure 2. In the first lot ripened, ethylene production remained at a very low level (0.009–0.03 ml. per kg./24 hour) for 7 days before a steady rise began. During the following 6 days there was a twenty-three-fold increase, followed by a sharp decline when the fruit became overripe. The curves for samples of fruit ripened at later dates are similar, except that the increase in ethylene production began immediately on transfer to the higher temperature.

Certain differences were observed in the trend of respiration during ripening of fruit withdrawn at different dates during the storage period. In the first sample, CO_2 output and O_2 intake remained at a low level for 7 days and then approximately doubled during the following 6 days. The peak in respiration coincided very closely with the peak in ethylene production. For the samples of fruit withdrawn at later dates there was a steady increase in the initial rates of CO_2 production and O_2 consumption, so that the sharp incline characteristic of the climacteric rise was of shorter duration. In the last sample of fruit ripened, a secondary increase in respiration occurred during the post-climacteric period. No increase in rate of ethylene production occurred during this final rise in CO_2 output and O_2 intake. KIDD and co-workers (**12**) have reported that total combustible volatiles of Conference pears do not increase during the secondary rise in respiration.

Table 1 presents the initial and maximum rates of evolution of CO_2 and ethylene for each lot of fruit ripened at the different dates during the storage period. The relative increases, calculated as maximum rate/initial rate, are also given.

The data of table 1 indicate certain well-defined trends for respiration and for ethylene production during storage and ripening. There is a progressive increase

in the initial rate of respiration for each lot of fruit when removed from cold storage to warm temperature. In the Bartletts this increase was from 11.61 to 25.90 ml. per kg.-hour, and in the Anjous from 9.45 to 18.23 ml. per kg.-hour. The initial rates of ethylene production likewise showed distinct indication of increasing. In Bartletts the range was from 0.49 to 4.39 ml. per kg.-day and in Anjous from 0.009 to 0.12 ml. per kg.-day. As shown in the table, the relative increases during the climacteric are much greater for ethylene production than for respiration. This may indicate that the two processes are not directly related. Additional evi-

TABLE 1

INITIAL AND MAXIMUM RATES OF CARBON DIOXIDE AND ETHYLENE PRODUCTION AT 20° C. AFTER DIFFERENT PERIODS OF STORAGE AT 0° C.

Months stored	Rate of respiration (ml. per kg.-hour)			Rate of ethylene production (ml. per kg.-day)		
	Initial	Maximum	Increase	Initial	Maximum	Increase
	Bartlett					
0	11.61	33.03	2.85	0.490	3.25	6.63
3	25.90	39.89	1.54	4.390	4.48	1.01
	Anjou					
2	9.45	18.79	1.99	0.009	0.700	78.80
4	15.76	20.72	1.31	0.010	0.726	72.60
5	16.03	19.89	1.24	0.065	0.570	8.77
6	15.60	21.00	1.35	0.105	0.761	7.25
7	18.23	23.27	1.28	0.120	0.782	6.52

dence in favor of this view is shown by the wide varietal differences in respiratory activity and ethylene production. The maximum rate of respiration for Bartlett pears is approximately double the rate for Anjous, yet the maximum rate of ethylene production is six or seven times greater in Bartletts than in Anjous.

The production of ethylene in relation to respiration during ripening of pears is distinctly different from that for apples and bananas as reported by NELSON (**17, 19**). He found that during the climacteric for these fruits, ethylene production decreased but increased again in the post-climacteric stage. Thus there was an inverse correlation between the emanation of ethylene and the production of CO_2. He suggested that ethylene was "consumed" during respiration and presented experimental evidence to that effect (**18**). If ethylene is metabolized during respiration of pears, the highest rate must occur when respiratory activity is low,

since the highest rates of ethylene production were found to coincide with the highest rates of respiration. No experimental evidence could be obtained, however, that ethylene is oxidized or otherwise metabolized by pears when respiratory activity is either high or low. Pure ethylene in small amounts was added to a desiccator containing Anjou pears in all stages of ripeness. There was an initial loss during the first 2 hours, probably due to adsorption of the gas on the glass walls and diffusion into the fruit; no further loss could be detected. The apparent discrepancies found between the emanation of ethylene and the respiration of pears cannot be explained on the assumption that ethylene is metabolized by the fruit.

No explanation is apparent at the present time for the differences in the relation of ethylene production to respiration of pears and apples. The time relation for the occurrence of the climacteric is different for the two fruits, and this fact may have some bearing on the discrepancy found. In apples (as well as in bananas) the climacteric precedes actual ripening of the fruit. In pears, however, ripening begins with the climacteric rise, and at the initial respiratory maximum the fruit is fully ripe.

Production of ethylene in relation to respiration under conditions of altered temperatures and oxygen concentrations

The data presented in the previous section have shown that ethylene production and respiration do not follow precisely parallel courses during ripening at 20° C. Since respiration can be altered by several experimental treatments, the production of ethylene under similar treatments was investigated.

The production of ethylene when fruit is maintained under anaerobic conditions was investigated first. For this experiment, three lots of Bartlett pears which had been in storage for 3 months were used. The fruit was kept in air for the first day. On the second day the air in the several desiccators was displaced with nitrogen, hydrogen, and helium, respectively. On the third day all lots were again transferred to air. Determinations of CO_2 and ethylene were made daily.

It is apparent from the results obtained (fig. 3) that the emanation of ethylene is not associated with the production of CO_2 under anaerobic conditions. Upon transfer of the fruit to oxygen-free atmospheres, ethylene production was either greatly retarded or totally inhibited. When transferred to air, emanation again took place. The production of CO_2 under aerobic and anaerobic conditions, however, changed very little.

This experiment was repeated the following season on newly-picked Bartlett pears. One lot of fruit was kept for 3 days in air, transferred to nitrogen on the fourth day, and then maintained in air for an additional 5 days. A second lot was kept continuously in air. The results are shown in figure 4.

The sample of fruit confined continuously in air showed the usual climacteric rise and increase in ethylene production followed by the sharp decline shown previously for pre-climacteric pears. The behavior of the treated lot, however, was decidedly different. On transferring the fruit from air to nitrogen, the rate of ethylene production declined below the amount quantitatively determinable. Respiration as measured by CO_2 showed very little change. On transfer from nitrogen to air, ethylene production increased to slightly above the maximum rate for the control fruit, and thereafter ethylene production in both lots declined at approximately the same rate. The production of CO_2, on the other hand, declined

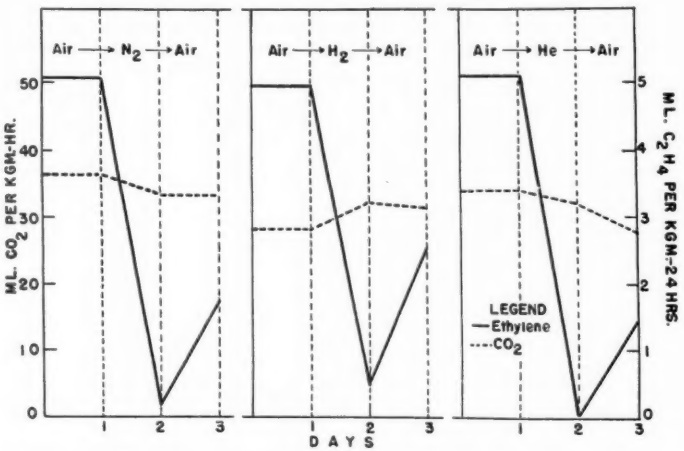


FIG. 3.—Comparative production of ethylene by Bartlett pears under aerobic and anaerobic conditions.

the first day following nitrogen treatment, then increased slightly before showing the senescent decline. The striking feature shown in this experiment is that subjecting the fruit to anaerobic conditions for 1 day prevented completion of the climacteric rise. In spite of this fact, ethylene production rose from zero to slightly above the maximum rate shown by the fruit held continuously in air. It has been possible, therefore, experimentally to decrease the magnitude of the climacteric without altering the usual trend of ethylene production.

The effect of temperature on respiration and ethylene production was also investigated. For these experiments Comice pears which had been kept in storage for 2 months were used. The fruit was brought directly from 0° C. storage, carefully sorted into five uniform lots, and then placed in constant temperature cabinets maintained at 0°, 10°, 20°, 30°, and 40° C., respectively. After the temperature of the fruit had reached the respective air temperature, it was placed in

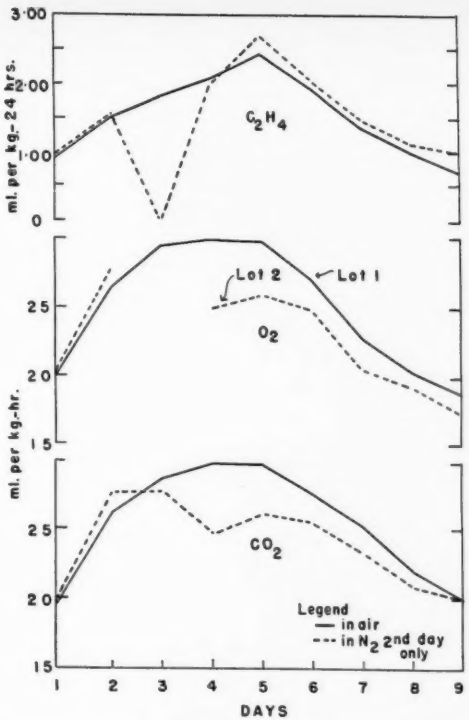


FIG. 4.—Effect of nitrogen storage of Bartlett pears on subsequent production of ethylene and respiration in air. Lot 1 held continuously in air at 20° C. Lot 2 transferred to nitrogen on second day but held continuously in air thereafter.

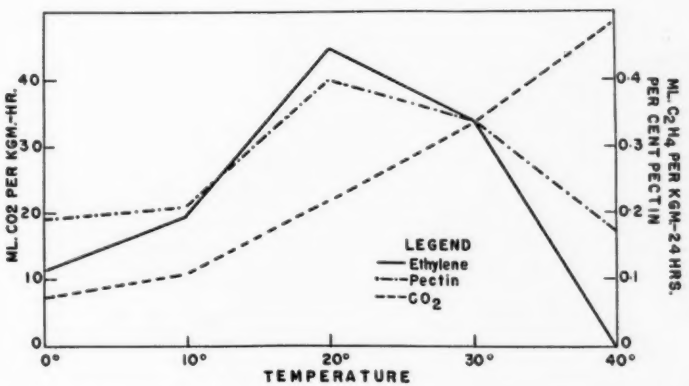


FIG. 5.—Effect of temperature on ethylene production, respiration, and pectin formation in Comice pears.

desiccators and supplied with oxygen as previously described. Determinations of CO_2 and ethylene were made after 24 hours. One-half of each lot was then used for the determination of the internal concentration of CO_2 and O_2. The remainder of the fruit was kept for an additional 5 days for soluble pectin analyses.

That changes in temperature do not affect ethylene production and respiration to the same degree is apparent from the data obtained (fig. 5). Between 0° and 20°, ethylene and CO_2 production both increased, the former reaching a maximum at the higher temperature. From 20° to 40°, CO_2 production continued to increase steadily but the rate of ethylene evolution declined sharply, reaching a zero value at 40°. The rate of soluble pectin formation also increased up to 20°, but declined thereafter. It is known from previous experiments (**21**) that the ripening of pears

TABLE 2

EFFECT OF TEMPERATURE ON CO_2 AND O_2 CONTENT OF COMICE PEARS

TEMPERATURE (° C.)	PERCENTAGE CO_2	PERCENTAGE O_2
0	9.99	26.61
10	18.56	16.19
20	23.33	12.62
30	30.50	6.57
40	27.50	4.51

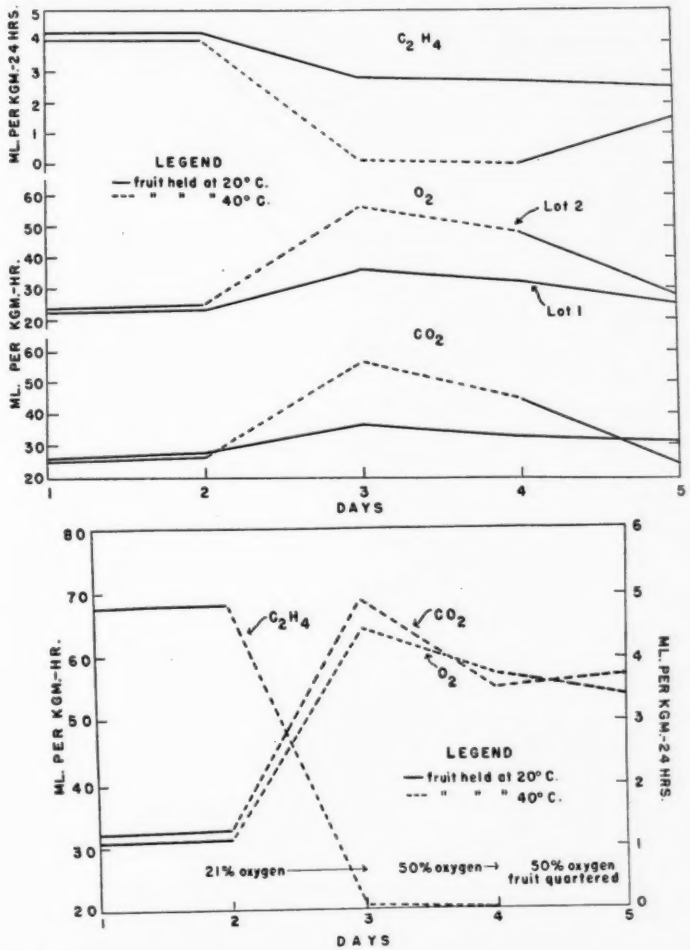
is retarded by high temperatures, but the significant fact shown in the present experiment is that ethylene production is similarly retarded.

The data presented in table 2 show that with increase in temperature there is an increase of CO_2 and a decrease of O_2 in the fruit tissues. From these data it could be assumed that at high temperatures, oxygen became a limiting factor so that no ethylene was produced. To investigate this possibility, the following experiments with Bartlett pears were carried out. One lot of fruit was maintained continuously at 20° during ripening. A second lot was kept for 2 days at 20°, then transferred to 40° for 2 days, and finally returned to 20° for 1 day. Oxygen consumption in addition to ethylene and CO_2 production was measured daily.

As shown in figure 6, ethylene production declined from approximately 4 ml. per kg.-day to zero on transfer of the fruit to the higher temperature. In contrast, respiration as measured by either CO_2 or O_2 greatly increased, the RQ being slightly greater than unity at 40° C. When transferred to 20°, emanation again occurred, showing that destruction of the ethylene precursor or enzyme system had not taken place.

In a second experiment the effect of increasing the concentration of oxygen in fruit held at 40° was investigated. The first day the fruit was confined in air.

The second day the same oxygen concentration was maintained, but the temperature was raised to 40°. On the following day the concentration of oxygen was in-



FIGS. 6, 7.—Fig. 6 (top), effect of temperature on ethylene production and respiration of Bartlett pears. Fig. 7 (bottom), effect of increasing oxygen tension of Bartlett pears held at 40° C. on ethylene production and respiration.

creased to 50 per cent. On the final day at the higher temperature the fruit was cut into quarters but retained in 50 per cent oxygen.

The data (fig. 7) show that increasing the oxygen concentration to 50 per cent

or increasing the absorptive surface by quartering the fruit had no effect on ethylene production at 40°. There was a small amount of ethylene found the first day on transfer to high temperature, but the production dropped to zero thereafter. Oxygen consumption as well as CO_2 production increased with rise in temperature, clearly demonstrating that aerobic respiration was taking place. In spite of this fact, no ethylene was produced. It could be assumed that probably the high content of CO_2 in the tissues inhibited ethylene production, or that ethylene was metabolized by the fruit when kept at high temperatures. Both these possibilities were investigated but with negative results. Ethylene production was found to be retarded but not inhibited in a mixture of 33 per cent CO_2 and 21 per cent O_2. Nor could any loss of ethylene be detected at 40° when small amounts of the gas were added to the desiccator according to the method used by NELSON (18).

Analysis of the gases extracted from pears kept at 40° showed no ethylene present. The addition of synthetic ethylene to fruit maintained at high temperatures, however, did not have any effect on ripening, the fruit remaining hard and green after 6 days' treatment. These data show that the mere presence of ethylene in the tissues is not sufficient to bring about ripening of the fruit under these conditions. Apparently the specific reaction from which ethylene is formed as a product must take place when the fruit ripens.

EFFECT OF H_2S AND HCN AS RESPIRATORY INHIBITORS

The data presented in the previous section strongly indicate that ethylene production is associated with a special oxidative system which is inhibited by high temperature. The amount of oxygen required for the functioning of this system apparently is smaller than could be detected or accurately measured by the methods used. Since certain of the oxidative enzymes are known to be inhibited by such substances as H_2S and HCN, response of the specific enzymes associated with respiration and ethylene production might differ in relation to these reagents. To investigate this possibility, Anjou pears in the post-climacteric stage were treated with H_2S and HCN in concentrations ranging from 0.1 to 4 per cent. The results obtained from these experiments have been inconclusive. Neither ethylene production nor oxygen consumption was affected by these reagents at the low concentrations found to be effective on other kinds of plant structures. At higher concentrations than those generally used, both oxygen uptake and ethylene production were partially inhibited. In one experiment a concentration of 4 per cent H_2S completely inhibited ethylene production and greatly retarded oxygen consumption. The fruit, however, showed severe injury after 2 days' exposure and did not ripen when treatment was terminated. The only conclusion that can be made at the present time from these data is that fruit in the post-

climacteric stage is not sensitive to the low concentrations of H_2S or HCN found to retard respiration in other kinds of tissues. It is possible that the reaction of enzymes to these substances is not the same at all stages of development of the fruit. MARSH and GODDARD (13) found the respiration of young carrot leaves to be inhibited by NaN_3, HCN, and CO, but when the leaves became older the respiration was not affected by any of these poisons.

Discussion

The quantitative data obtained during the course of this investigation show that the production of ethylene is a definite part of the metabolism of mature pears. If the formation of ethylene resulted from a chance reaction, then it would be expected that emanation of the gas would be erratic and follow no well-defined trend. This has not been found to be the case. Each sample of fruit ripened at 20° C. showed the same general trend of production—an initial increase during the climacteric rise, the attainment of a maximum rate, and a decline during the senescent period. Moreover, each of the varieties studied over an extended period was found to have a definite, characteristic maximum rate of production, and this value varied but little among the different lots of fruit ripened. It may be concluded that the formation of ethylene is an integral part of the metabolic activities of mature pears. This fact is significant in that it forms the basis for the consideration of ethylene production in relation to other phases of fruit metabolism.

Determinations of the rate of ethylene production and of respiration were carried out simultaneously on the same samples of fruit. The results show that the two processes follow somewhat the same general trend during ripening, but no indications that ethylene production is directly related to respiration were found. In the first place, the relative increase in ethylene production during the climacteric rise is out of all proportion to the increase in the rate of respiration as measured by either CO_2 or O_2. Thus, respiratory activity approximately doubles during this initial stage of ripening, while ethylene production increases seven- to eighty-fold, depending upon the variety and length of time it has been held in cold storage prior to ripening. During the post-climacteric stage, the emanation of ethylene declines rapidly, but respiration, especially as measured by CO_2, declines at a much slower rate. Moreover, the last sample of Anjou pears ripened late in the storage season showed a secondary rise in respiration during senescence, but this was not accompanied by a similar rise in ethylene production. Additional evidence that the production of ethylene and respiration are not necessarily directly related is shown by the wide differences in the ratio CO_2/C_2H_4 for the two varieties of fruit studied. During the climacteric, Bartlett pears respire at approximately double the rate for Anjou, yet the rate of ethylene production is six or seven times greater in the former variety.

When pears were subjected to experimental alterations in oxygen tension and temperature, ethylene production and respiration were found to become even more widely separated. In oxygen-free atmospheres the formation of ethylene was either greatly retarded or entirely inhibited. In contrast, the rate of CO_2 evolution was changed but little by this treatment, showing that the formation of ethylene is not correlated with CO_2 production under anaerobic conditions.

Further evidence that ethylene production and respiration are not directly related is shown by their difference in response to high temperatures. From 0° to 20° C., the Q_{10} for the two processes was found to be approximately the same. At higher temperatures, however, the responses were entirely different. Respiratory activity continued to rise with increase in temperature beyond 20°. On the contrary, ethylene production declined rapidly, and at 40° C. no emanation could be quantitatively detected. That oxygen supply is not a limiting factor under these conditions has been shown. Thus, even though the rate of aerobic respiration was greatly accelerated at 40°, the production of ethylene was entirely inhibited, apparently by the inhibition of the reactions associated with its formation.

Since there appears to be no positive correlation between ethylene production and respiration under either anaerobic or aerobic conditions, it seems necessary to postulate that the formation of ethylene is not directly associated with that phase of respiration responsible for the major portion of the CO_2 produced and O_2 consumed. It can by no means be assumed, however, that the method of formation of ethylene is to be considered as entirely separate and distinct from the respiratory mechanism in fruits. Respiration in fruits, as in certain other plant structures (**6, 22**), may consist of several distinct but interrelated systems—some requiring oxygen, others not; but each contributes to the total amount of CO_2 being measured. According to this concept, the total amounts of O_2 consumed or CO_2 produced by the fruit will not necessarily be correlated with the activity of each individual system of the respiratory complex.

Summary

1. A quantitative study of ethylene production in relation to the respiration of pears has been made. The emanation of ethylene in air at 20° C., as well as at higher and lower temperatures in modified atmospheres, has been studied.

2. In fruit in air at 20° C. the rate of ethylene production increases during the climacteric rise in respiration, reaches a peak at the respiratory climax, then declines during the post-climacteric period. During the climacteric rise, ethylene production increases seven- to eighty-fold, while rate of respiration approximately doubles.

3. Each variety was found to have a characteristic maximum rate of production. The maximum rate for Bartlett, a variety which maintains its capacity to

ripen for only a short period of time when kept at a storage temperature of 0° C., is 3.25–4.48 ml. per kg.-day. The maximum rate for Anjou, a variety which maintains its capacity to ripen for a long period of time when kept at cold storage temperatures, is 0.57–0.78 ml. per kg.-day. The maximum rate of respiration for Bartlett is approximately double that for Anjou.

4. Under anaerobic conditions, the production of ethylene is either greatly retarded or entirely inhibited. In the fruits used for these experiments, there was found but little difference in the production of CO_2 under aerobic and anaerobic conditions.

5. The maximum rate of ethylene production occurs at 20° C. At higher temperatures production decreases and is totally inhibited at 40° C. Respiratory activity as measured by either CO_2 production or O_2 consumption is greatly accelerated between 20° and 40° C.

The writer expresses his thanks to Dr. C. A. SHULL and others of the botany department of the University of Chicago for their assistance, and to Professor F. C. REIMER, Superintendent of the Southern Oregon Experiment Station, for supplying the fruit used in this investigation.

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GAMETOPHYTES OF MARATTIA SAMBUCINA AND MACROGLOSSUM SMITHII

ALMA G. STOKEY

(WITH THIRTY FIGURES)

Introduction

The sporophytes of the Marattiaceae show considerable range in form and size, but the gametophytes are decidedly uniform. They are sufficiently different from the gametophytes of the leptosporangiate ferns as to be easily recognized. Descriptions varying in their fullness have been given of the gametophytes of the following species: *Angiopteris evecta* by LUERSSEN (13), FARMER (8), SCHMELZEISEN (14), and HAUPT (9); *A. pruinosa* and *Angiopteris* sp. by JONKMAN (11, 12); *Macroglossum alidae* by CAMPBELL (7); *Marattia cicutaefolia* by LUERSSEN (13); *M. fraxinea*, *M. weinmannifolia*, *M. kaulfussii*, and *M. verschaffeltiana* by JONKMAN (11, 12); *M. douglasii* by CAMPBELL (3); *M. alata* by SCHMELZEISEN (14); *Danaea simplicifolia* by BREBNER (2); *D. jenmanii*, *D. elliptica*, and *D. jamaicensis* by CAMPBELL (5, 6); and *Kaulfussia aesculifolia* by CAMPBELL (4).

Material and methods

The spores of *Marattia sambucina* Bl. were collected by the writer from plants growing wild in a rich moist ravine in the forest near the Botanical Garden at Tjibodas, Java, in June, 1931, and July, 1937. The set of cultures started in 1931 grew for 2 years; the second set, started in Java in 1937, are still growing at Mount Holyoke College. The work on *Macroglossum smithii* (Rac.) Campbell is based on a set of cultures from spores collected in 1931 from a plant in the Botanical Gardens at Buitenzorg, which is doubtless the plant to which CAMPBELL refers in his discussion of the relation of *M. smithii* to *M. alidae* (7). Germination stages were obtained on distilled water and later stages on peat by methods described previously (15). Fresh prothalli were used for the study of germination stages, habit, rhizoids, external aspect, and distribution of sex organs. A weak chromo-acetic acid with a few drops of osmic acid gave the most satisfactory results in killing and fixing, but formalin-acetic acid and a modified Navashin's fluid were also good. It is more difficult to get satisfactory preparations of the prothalli of the Marattiaceae than of other families of ferns, probably because of the heavily cutinized cells surrounding the delicate internal tissues.

The early work was carried out in the Treub Laboratorium, Buitenzorg, Java.

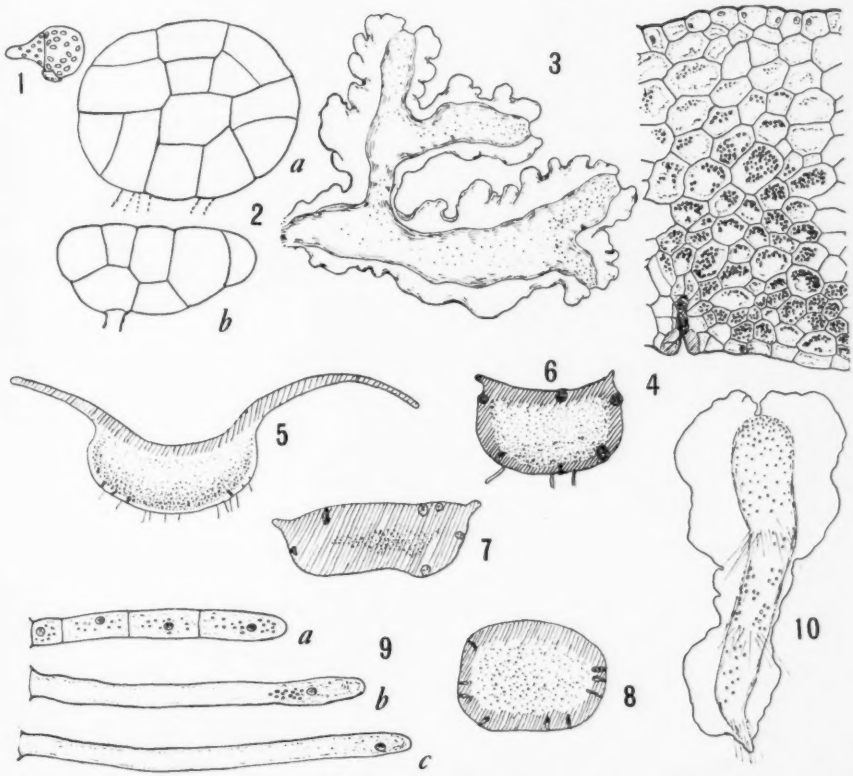
The cultures were brought to the United States and the work continued in the laboratories of Mount Holyoke College and the Marine Biological Laboratory, Woods Hole, Massachusetts.

Observations

Spore germination and vegetative stages

The spores of *Marattia sambucina* are pale yellowish tan, bilateral and elongated, with a lengthwise ridge. The wall is slightly thickened and roughened. No radiate spores were found, but there were many spores—neither elongated nor thickened—which appeared to be immature and which failed to germinate. The spores become distinctly green before the spore coat ruptures. The cracking of the coat was seen 16 days after planting. There was good germination and sufficient growth 25 days after planting to give the peat the characteristic dark green color of the Marattiaceae. There is great enlargement of the primary cell before the first division occurs; this usually cuts off the first rhizoid-bearing cell, although four or five cells may form before a rhizoid develops. The chloroplasts are conspicuous in the rhizoid and may persist for considerable time, not only in the primary rhizoid but in those formed much later (figs. 1, 9*a*, *b*). The next divisions result in a plate, and the plate develops into a mass. The division in the third plane may begin when there are only four cells, or it may not occur until later. The early thickening may not involve all the cells, as in figure 2, in which only the basal portion of the thallus is two cells thick. No case was seen in which there was a filamentous stage at germination, although there were many irregularities. The apical cell may appear just after the quadrant stage but usually not until later. The next stages lead gradually to the development of a heart-shaped thallus. A midrib is formed which is not necessarily continuous with the thickened base. The wings are two cells thick, except for the margin which is one cell thick and the region near the midrib which may be heavier. Like all the gametophytes of the Marattiaceae which have been described, they are notable for their dark green color. Crowded prothalli may be distinctly slender and have a blunt tip. No filamentous ameristic prothalli were found. When growth is continued for several years under varying conditions, there may be periods in which rib development is dominant and little or no wing is formed (figs. 6, 7, 8); but under conditions which are probably more favorable there is development of wing as well as midrib (fig. 5). The wings are usually flat, and while they may become irregular they are not ruffled as in *Gleichenia*. Branching is not uncommon in gametophytes several years old; and old gametophytes, branched or unbranched, usually show irregular margins. The branching prothallus in figure 3 was 27 months old and had four apices, three of which were producing archegonia. If fertilization does not occur, the gametophytes under culture conditions may grow indefinitely and in 3 or 4 years may attain a length of 25–35

mm. The structure of a midrib in a region in which there were many old archegonia is shown in figure 4. As is often the case in old prothallia, there is abundant storage of starch. The difference in size which commonly exists between the superficial and the internal cells may be noted. The layer of heavily cutinized cells on both dorsal



FIGS. 1–10.—Figs. 1–9, *Marattia sambucina:* Fig. 1, stage shortly after germination of spore. Fig. 2, two views of young prothallus, posterior portion two cells thick. Fig. 3, prothallus 27 months old. Fig. 4, cross section of midrib containing starch. Figs. 5–8, cross sections of prothalli through portions showing variation in midrib and wing development; shading indicates chlorophyll-bearing cells; stippling indicates starch. Fig. 9, young rhizoids from prothalli 4 years old; *a* and *b* contain disintegrating chloroplasts. Fig. 10, *Macroglossum smithii*, prothallus 13 months old bearing many archegonia and antheridia.

and ventral surfaces seems to constitute a physiological epidermis. Figure 5 shows a section cut from a fresh prothallus in which the wings and upper parts of the midrib were deep green, but the rest of the midrib was colorless and contained much starch, particularly in the lower layers of cells. The midrib may be green through-

out (fig. 7), only the surface layers may be green (figs. 6, 8), or only the upper layer (fig. 5). Figure 7 shows a thallus in which the starch was stored in the center, in cells smaller than those of the surrounding tissue, which were formed by secondary division of the large internal cells.

The rhizoids of *M. sambucina* are colorless, long, stout, and cutinized. In general they are limited to the midrib, and the production may be intermittent. A few old gametophytes were found to have septate rhizoids which were truly multicellular, with a nucleus in each cell (fig. 9*a*). The multicellular rhizoids consist of two to five cells and were found in the tufts of unicellular rhizoids, but were always in a minority. The production of septate rhizoids was not of rare occurrence in the old gametophytes examined but was not common enough to be considered characteristic. Young rhizoids—both unicellular and multicellular—containing chloroplasts were found on prothalli 3–4 years old (fig. 9*a*, *b*).

The presence of an endophytic fungus has been reported for the gametophytes of the Marattiaceae collected in the open. Naturally no fungus was found in the gametophytes under investigation, which were grown on sterilized peat. Their vigorous growth and normal development support the view that the fungus is of little or no importance in the life of the gametophyte.

Regeneration occurs vigorously under certain conditions. The margin or even the surface of an old thallus may develop such a large number of regenerated growths as to suggest a rosette. The buds usually form a quadrant, much like a young thallus growing from a spore, and then—if space permits—the development proceeds as in a young prothallus (figs. 26, 27). Crowding prevents symmetrical development in many cases. Thickening of the posterior portion of the new thallus may occur early, with the anterior portion remaining one cell thick for a considerable time and sometimes broadening into a fanlike form by marginal growth. The regenerated branch may give rise to other regenerated branches before separation from the parent thallus takes place. In general, the rhizoids develop later than on similar structures in the Polypodiaceae. Small antheridia may appear on the young regenerated growths before separation from the parent thallus is complete (fig. 30).

The spores of *Macroglossum smithii* are of the radiate type and are pale in color. No elongated spores were seen. Germination was abundant. The germination and early stages were followed less closely than those of *Marattia sambucina*, but all stages seen suggest the same range in form and the same type of development from germination of the spore until maturity of the sex organs. The gametophytes have the deep green color characteristic of the family. The wings, while not perfectly regular, are not curled or crisped but usually lie flat. The midrib may become very heavy, and the proportion of wing to midrib varies under certain undetermined conditions, as in *M. sambucina* (fig. 10). Unlike *Marattia*, the wings tended to

remain one cell thick, as CAMPBELL noted for *Macroglossum alidae;* but no prothallia were in culture as long as those of *Marattia*. The rhizoids are long and colorless, holding the thallus close to the ground. No multicellular rhizoids were found.

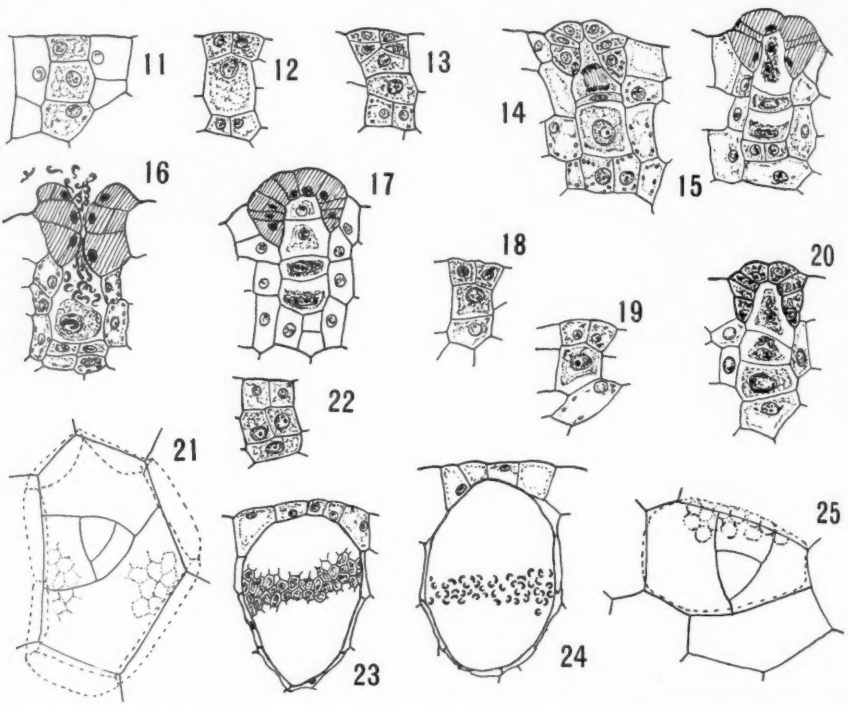
REPRODUCTIVE STRUCTURES

Antheridia were abundant on prothalli of *Macroglossum* 5 months old but did not appear on *Marattia* until the prothalli were 8 months old, and they were never quite as abundant as on *Macroglossum*. They appear first on the ventral side but may soon be found on the dorsal side as well. Archegonia were found on both species when the gametophytes were about 1 year old. As all the cultures were started in June or July and brought to the United States in September, it is possible that the weaker light of late autumn and early winter retarded the development of the prothalli and delayed the formation of sex organs. The production of antheridia usually continues throughout the life of the gametophyte, but after the production of archegonia begins the antheridia may develop chiefly on the dorsal surface. Antheridia may continue to form when conditions become unfavorable for the formation of archegonia.

The development of the antheridium of the two species is apparently just like that described for other species of the Marattiaceae. It is initiated in the primary antheridial cell by a periclinal division, which separates the primary cover cell from the primary spermatogenous cell (fig. 22). In the formation of small or medium-sized antheridia the primary cover cell undergoes three divisions, and four in the case of large antheridia, forming in the center a triangular opercular cell (figs. 21, 25). Secondary divisions may occur in one or more of the cover cells surrounding the opercular cell. There is a great range in the size of mature antheridia. Antheridia on regenerated branches may show only 15 to 30 sperms in a median section, but on vigorous young gametophytes the number is likely to range from 50 to 80, while on vigorous large prothalli the range may be from 140 to 170, a number considerably larger than that usually credited to the Marattiaceae. The antheridia shown in figures 23 and 24 are not exceptional for *Macroglossum smithii*, and antheridia equally large or larger may be found on *Marattia sambucina*. The spermatocyte mass is surrounded by a mantle or jacket layer cut off from the surrounding cells—except on the side adjoining the cover cells (figs. 21, 23, 24). In longitudinal section it can be seen that the mantle layer is sometimes incomplete.

Stages in the development of the archegonium of *Marattia sambucina* are given in figures 11–16. In the development of the archegonium of *M. sambucina* a basal cell was found to be a characteristic feature. In *Macroglossum smithii*, on the contrary, the archegonium more frequently develops without a basal cell (fig. 19), although such a cell may form occasionally (fig. 18). The neck and axial row are

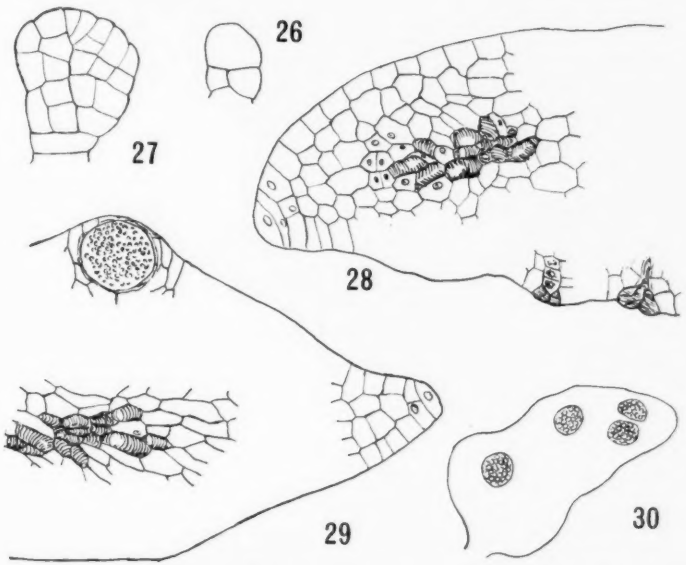
formed by the usual divisions of the primary cells, as given in detail by HAUPT (9). The neck of both species, as in other members of the family, is very short and scarcely projects above the surface. The flatness is a result of the form and size of the cells rather than of their number. The neck consists of four rows of cells, and



FIGS. 11–25.—Figs. 11–17, *Marattia sambucina:* Figs. 11–16, series in development of archegonium. Fig. 17, archegonium with two neck canal cells. Figs. 18–24, *Macroglossum smithii:* Fig. 18, early stage of archegonium with basal cell. Fig. 19, without basal cell. Fig. 20, mature archegonium. Fig. 21, surface view of antheridium with four divisions in primary cover cell; jacket layer indicated by dotted lines. Fig. 22, early stage of antheridium. Figs. 23, 24, longisection of antheridia from gametophytes 16 months old. Fig. 25, *Marattia sambucina*, surface view of antheridium with three divisions in primary cover cell.

each row consists usually of three cells; but the middle cell frequently divides, forming two cells which are apt to be flatter than the outer and inner cells. Occasionally there are five cells in a row (fig. 17). The number of cells is not always the same in all rows, but no consistent pattern in the variation was found. The walls of the neck cells become modified as the archegonium matures, and the cell contents undergo changes such that stained preparations of even the early stages may show coarse granules, and those of the later stages may show little or no differentia-

tion except for the nucleus. The axial row is short and more uniform in diameter than is usual in ferns. The egg is relatively small and the ventral canal cell relatively large, almost the size of the egg (figs. 15, 17, 20). A thin ventral canal cell is unusual (fig. 14). The mature archegonium usually shows two nuclei in the neck canal; but in several gametophytes of *Marattia*, archegonia were found in which there was a definite wall in the neck canal, forming two neck canal cells (fig. 17). In some cases the presence of two distinct protoplasts—instead of the customary



FIGS. 26–30.—*Marattia sambucina:* Figs. 26, 27, young regenerated branches. Figs. 28, 29, apogamy Fig. 30, regenerated branch bearing small antheridia.

single mass in the neck—indicated that a delicate wall had been present but had disappeared or was not revealed in the preparation. Fewer preparations were made of *Macroglossum*, and only one case was found with two neck canal cells. The jacket layer of nutritive cells around the archegonium venter is a conspicuous feature in leptosporangiate ferns but is less prominent in the Marattiaceae. In both species under investigation the jacket layer was late in development and never was as prominent as in other ferns. The basal cell, when present, divides early, and the resulting cells form the most conspicuous part of the jacket layer. Since in *M. smithii* the basal cell is usually lacking, the jacket layer is more belated than in *Marattia sambucina* and is even less conspicuous. At the time of fertilization there is usually a complete layer, but it may consist of so few cells that not

more than six will be seen in longitudinal section. The contents of the jacket cells are less dense than in leptosporangiate ferns, and the cells are more or less tabular in form rather than cubical (figs. 15–17). In both *Marattia* and *Macroglossum*, archegonia were found into which large numbers of sperms had penetrated (fig. 16). As might be expected, the robust gametophytes of these ferns can support two sporophytes for considerably longer than the more delicate gametophytes of the leptosporangiates. In the case of branched prothalli, the branches act as independent units, and even on unbranched prothalli two sporophytes may continue to grow for considerably longer than would be the case in other families of ferns.

Apogamy

Two cases of apogamy were found in *Marattia sambucina*, both consisting in the development of tracheids in the thallus. A gametophyte 27 months old had produced both antheridia and archegonia of normal appearance (fig. 28). In this thallus there were two distinct masses of tracheids found in different sequences of sections. The gametophyte (fig. 28) was more than 3 years old and had produced many apparently normal antheridia and archegonia. These gametophytes, like all the others, had been grown on peat with a moderate degree of uniformity of moisture, in a glass culture dish with a high degree of humidity, and were exposed to the light in a north window.

Discussion

The following may be considered distinguishing features of the gametophytes of the Marattiaceae: germination of the spore into a plate and then into a mass; slow development and relatively large size and thickness of the thallus; deep green color; rhizoids which are colorless in most species and which may be septate; imbedded antheridium with a large output of sperms; a short broad archegonium with a neck which scarcely projects and with a poorly developed jacket layer.

The filamentous stage at germination of the spore, which is so common in higher ferns, is extremely rare but has been reported by Jonkman (**11, 12**). The failure to develop a filament under adverse conditions, particularly of light (which induces it in most ferns), indicates a lack of plasticity which may be a decided handicap. The deep green color of the gametophyte is sufficiently uniform as to be distinctive of the family. The shade of green, combined with the large size and unusual thickness of the thallus, gives the gametophyte the appearance of a liverwort, with the colorless rhizoids contributing to the resemblance. Jonkman (**11, 12**), who worked on four species of *Marattia* and two of *Angiopteris*, states that the rhizoids are colorless and do not become brown, even on very old prothalli. Campbell (**4**) describes the rhizoids of *Kaulfussia* as being stout and thick with colorless walls, but he states that the rhizoids of *Danaea* and also of *Marattia* and *Angiopteris* are

brown in color (6). SCHMELZEISEN (14) speaks of the many chloroplasts in the rhizoids of *Angiopteris evecta*. He states that the rhizoids arise by the differentiation of ordinary prothallial cells, and that there are all degrees of differentiation between the rhizoid and the assimilating cell. Colorless rhizoids occur in many if not in most species of the Marattiaceae but are exceptional in other families of ferns. In the leptosporangiate ferns, as a rule, the rhizoids are colorless only in the young stage or on very young gametophytes, while on the mature gametophytes the range in color is from the pale tan of many species of the Polypodiaceae to a distinct brown such as is found in the Gleicheniaceae and the Hymenophyllaceae. The presence of conspicuous chloroplasts in the young rhizoids of young gametophytes of ferns with chlorophyll-bearing spores, such as those of *Osmunda*, has often been noted; but they may also occur in species without chlorophyll in the spore, as in many species of *Gleichenia*. In the higher ferns, except in the species with green spores, the chloroplast-bearing stage is very short if it occurs at all. The multicellular rhizoids which occur occasionally in *Marattia sambucina* are characteristic of the four species of *Danaea* which have been investigated (2, 5, 6). The high development of cutin which is characteristic of the superficial cells of the thallus is also characteristic of the rhizoids of the Marattiaceae. In general, the characteristics of the rhizoids of the Marattiaceae—prevalence of colorless rhizoids, persistence of chloroplasts, tendency to septation, and heavy cutinization—indicate that they are less highly differentiated from the other cells of the thallus and are less highly specialized than are the rhizoids of the leptosporangiate ferns.

The tendency for the thallus under certain undetermined conditions to develop as a subterete structure may throw some light on the origin of the Ophioglossaceae. The storage of starch is probably related to the relatively long life of the gametophyte of the Marattiaceae in contrast to that of higher ferns.

The archegonium is peculiar in the deeply set neck with only a slight projection, since in both the Ophioglossaceae and the leptosporangiate ferns the neck projects distinctly, even when—as in the Hymenophyllaceae—there are only four cells in a row. In regard to the ventral canal cell the family shows the two extremes, from the very small short-lived cell in *Danaea* to the condition in the other genera in which the ventral canal cell is much larger in proportion to the egg than in the leptosporangiate ferns. A wall between the neck canal nuclei has been reported for the Marattiaceae more frequently than for any other family. Within the family there is variation in the presence or absence of a basal cell, but the character does not appear to be consistent, even in a single species. The relatively slow and weak development of the jacket layer, noted first in the work of JONKMAN (11, 12), may be a handicap if it affects the early development of the embryo.

Apogamy has been reported for four families of the leptosporangiate ferns, but the only case known for the eusporangiates is in *Botrychium virginianum* described

by JEFFREY (10). In *Botrychium*, as in *Marattia*, it consisted in the formation of tracheids in the gametophyte. BEYERLE (1) reported apospory in *M. alata* consisting in the production experimentally of rhizoids on primary leaves.

Summary

1. Germination of the spores of *Marattia sambucina* and *Macroglossum smithii* results first in a plate and then in a mass. The gametophytes are dark green from the very first, but the old gametophytes may show a pale tissue in the midrib—which may contain a large amount of starch. The rhizoids of both species are colorless; multicellular rhizoids are produced occasionally by *Marattia sambucina*.

2. The antheridia show wide range in size, from those on regenerated branches which may show 15–30 sperms in median section to those on vigorous gametophytes 1–4 years old which may show 140–170 sperms.

3. The archegonium of both species is short and broad, projecting but slightly beyond the surface. The two nuclei of the neck canal are usually not separated by a wall, but occasionally a wall is formed. The ventral canal cell is large. The jacket layer consists of a few tabular cells which are slow in developing and not highly differentiated before fertilization.

4. Apogamy, shown in the development of tracheids, was found in two gametophytes of *Marattia sambucina* which had borne both antheridia and archegonia.

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AUXINS IN SOME AMERICAN SOILS

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Since auxins have been found in plant tissues it is reasonable to suppose that they may persist through decomposition stages of organic matter and become a part of the soil, or perhaps they may be synthesized during the decomposition process. The report presented here deals with the extraction of auxin from some virgin American soils and its relation to other soil characteristics. What this means in relation to plant growth is left for future consideration.

In this investigation the standard *Avena* test was used to measure the auxin extracted from the soils. The results are expressed as indoleacetic acid equivalents. This is an arbitrary term and does not imply that indoleacetic acid was the auxin extracted from the soil.

Various methods have been used for auxin extraction from different kinds of substances, but freshly purified peroxide-free ethyl ether has been a popular solvent. This background of experience was utilized in the development of a method adapted to extraction of soil auxins. The surface horizons of Cecil, Russell, and Barnes soils were used for a considerable number of preliminary experiments to find a satisfactory way of extracting comparable quantities of auxin. A résumé of these various tests follows:

1. Ether or alcohol used alone extracted little or no auxin, at least from acid soils.

2. Acidifying a soil-water mixture to a pH of 4.5 with hydrochloric or acetic acid, with or without refluxing, on an electric hotplate gave no auxin in the water-ether extract.

3. Mixtures of ether and water extracted more auxin from acid soils than either solvent alone, but approximately the same amount was extracted by water as by ether-water mixtures when the pH was slightly alkaline.

4. Water extraction at pH 7–8 formed the basis of the method adopted. This method is as follows.

One hundred gm. of air-dry soil in an 800-cc. beaker was treated with 200 cc. of water and enough limewater added to produce a final pH of about 7.5. About 30 per cent more limewater was needed than that required to hold such a pH for the first 2 hours, because of the slow reaction with soil acids. Water was then added to make the total volume of liquid 400 cc. After standing with occasional

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stirring for 48 hours, the total extraction period, the suspension was filtered on a Büchner funnel through no. 1 Whatman filter paper, and the filtrate evaporated to dryness in a casserole over a steambath. Twelve cc. of water was then added and the walls carefully policed down. This solution was transferred to a 250-cc. wide-mouthed Erlenmeyer flask and 120 cc. of ether added; the flask was stoppered, shaken at intervals, and stored in an icebox over night.

TABLE 1

RELATION OF AUXIN TO OTHER PROPERTIES IN VIRGIN SOILS

Name and location of soil*	Laboratory no.	Horizon	Depth (inches)	pH	Organic matter content† (%)	Indoleacetic equivalents in micrograms per kg. of soil	
						Sample 1	Sample 2
Barnes loam, South Dakota	C2929	A	0–9	6.9	5.98	0.165	0.134
	C2930	B_1	9–17	7.1	2.36	0.161	0.169
Brookston silty clay loam, Indiana	C4043	A_1	0–6	6.8	10.42	0.112	0.113
	C4044	A_2	6–20	6.4	2.67	0.148	0.196
Carrington loam, Iowa	C2916	A_1	0–3	5.5	5.57	0.106	0.141
	C2917	A_2	3–13	5.2	3.43	0.122	0.154
Miami silt loam, Indiana	C4060	A_1	0–2	6.3	6.22	0.084	.....
	C4061	A_2	2–5	5.9	3.60	0.034	.....
Russell silt loam, Indiana	C3174	A_1	0–2	5.8	6.26	0.125	0.118
	C3175	A_2	2–8	4.5	2.94	0.000	0.039
Hagerstown silty clay loam, Pennsylvania	C 798	A_1	0–2	4.5	11.04	0.043	0.068
	C 799	A_2	2–8	4.8	2.53	0.000	0.026
Hillsdale fine sandy loam, Michigan	C4031	A_1	0–3	7.5	4.28	0.118	0.072
	C4032	A_2	3–9	7.5	0.72	0.000	.....
Decatur silty clay loam, Georgia	C7178	A	0–12	6.3	4.05	0.132	0.100
	C7179	B_1	12–36	5.7	0.46	0.000	0.026
Cecil fine sandy loam, South Carolina	C7173	A_1	0–2	6.1	4.16	0.069	0.099
	C7174	A_2	2–8	5.1	0.85	0.000	0.067
Georgeville silt loam, North Carolina	C7186	A_2	½–5	4.5	3.44	0.097	0.092
	C7187	B_1	8–28	4.7	0.23	0.000	0.000
Norfolk fine sandy loam, North Carolina	C7190	A_1	0–2	4.4	10.52	0.038	.....
	C7191	A_2	2–13	5.3	0.43	0.000	0.000

*Soils arranged in order of approximate native fertility. † Determined by combustion, $CO_2 \times 0.471$.

The following day the ether was carefully decanted from the solution into another Erlenmeyer flask of similar size and evaporated on a water-bath to 3–5 cc. This volume of ether was then further evaporated drop-wise by means of a pipette on 0.3 cc. of 1.5 per cent agar contained in a 5-cc. shell vial. During evaporation, the vial was suspended in a rapidly boiling water-bath. The flask was carefully rinsed twice with 2-cc. portions of fresh ether and these likewise evaporated on the agar. Then the walls of the shell vial were carefully washed down with fresh ether. By this procedure auxin from a 48-hour water extract of soil was concentrated in 0.3 cc. of 1.5 per cent agar. This volume of agar was molded into

twelve blocks, suitable for auxin determination by the *Avena* test. Inasmuch as the amounts of auxin obtained during this procedure caused *Avena* curvatures that were less than the maximum angle (30°), auxin determinations at dilutions of the original extract were not made.

The general characteristics of most of the soils used have been summarized (19). The soils are typical specimens of eleven soil series representing four of the great soil groups. Each soil was air-dried and passed through a 2-mm. sieve. The soils had been stored for periods varying from 3 months to 4 years. Data for these soils are given in table 1. In table 2 more detailed data obtained in the *Avena* test are given. While the soils are listed in the order of their approximate fertility, this listing should be considered as having only general significance. These tables, together with the preliminary results, not given in detail, show that the extractable auxin content tends to decrease with increasing soil acidity and that surface and second horizons of soils of excellent native fertility tend to have higher extractable auxin contents than do the second horizons of less fertile soils.

It is recognized that determination of the auxin content of soils by the procedure described does not possess the same degree of precision as is involved in the determination of most of the inorganic chemical constituents. It is not known what part of the auxin present is extracted by the means used, nor is it known to what extent—if any—auxin is created or destroyed by the extraction process which is carried out under nonsterile conditions. The important fact is that fairly concordant duplicate determinations were obtained when extractions were made at different times and evaluated with different sets of oat plants separately standardized as to sensitivity to auxin.

These data are in accord with those obtained by PARKER-RHODES (12), whose study of cultivated soils constitutes the only previous work on determination of soil auxin. He used a root-hair plasmolysis technique to estimate the amounts of auxin. In terms of indoleacetic acid, concentrations of 10^{-9} to 10^{-10} moles per kilogram of soil are reported. He suggests the auxin from soil has certain characteristics of indoleacetic acid, but at present the chemical compound or compounds acting as soil auxin(s) must be regarded as unknown.

From auxin determinations of soil microörganisms, ROBERTS and ROBERTS (13) conclude that the soil flora is potentially capable of producing appreciable amounts of auxin. The work of KÖGL and KOSTERMANS (6), THIMANN (16), and others indicates that the auxin produced by many of these microörganisms is indoleacetic acid.

It has been reported that 10^{-9} molar indoleacetic acid stimulates root growth of several kinds of plants (1, 2, 17). Since this optimum concentration of auxin for root growth is found only in the fertile soils, it may be one of the factors in some way contributing to their fertility. Furthermore, the preliminary experi-

TABLE 2

CURVATURE OF AVENA COLEOPTILES IN RELATION TO AUXIN EXTRACTED FROM VIRGIN SOILS (AS DEGREES, AND AS MICROGRAMS OF INDOLEACETIC ACID EQUIVALENTS PER KG. OF SOIL, BRACKETED NUMBERS)

Soil tested	Horizon	Depth (inches)	Date of Avena test							
			10/4	10/6	10/7	10/9	10/10	10/11	10/16	10/17
Barnes, South Dakota	A	0–9	.....	.....	22.6 (0.134)	.....	.....	17.6 (0.165)	.....	.....
	B_1	9–17	.....	.....	18.5 (0.109)	.....	.....	18.0 (0.169)	20.1 (0.161)	.....
Brookston, Indiana	A_1	0–6	17.0 (0.112)	.....	.....	11.3 (0.113)	.....	.....	18.3 (0.147)	.....
	A_2	6–20	.....	24.4 (0.196)	.....	14.8 (0.148)	.....	.....	25.2 (0.202)	.....
Carrington, Iowa	A_1	0–3	.....	.....	17.9 (0.106)	.....	.....	.....	17.6 (0.141)	.....
	A_2	3–13	.....	.....	20.5 (0.122)	.....	24.7 (0.172)	.....	.....	22.0 (0.154)
Miami, Indiana	A_1	0–2	.....	.....	.....	.....	.....	.....	.....	12.1 (0.084)
	A_2	2–5	.....	.....	.....	.....	.....	.....	.....	4.9 (0.034)
Russell, Indiana	A_1	0–2	.....	.....	20.0 (0.118)	.....	.....	.....	.....	17.9 (0.125)
	A_2	2–8	.....	.....	0.00 (0.000)	.....	.....	4.2 (0.039)	.....	.....
Hagerstown, Pennsylvania	A_1	0–2	6.5 (0.043)	.....	.....	.....	9.6 (0.068)	.....	.....	.....
	A_2	2–8	0.00 (0.000)	.....	.....	.....	3.7 (0.026)	.....	.....	.....
Hillsdale, Michigan	A_1	0–3	.....	8.8 (0.072)	.....	11.8 (0.118)	.....	.....	.....	.....
	A_2	3–9	.....	0.0 (0.000)	.....	.....	.....	.....	.....	.....
Decatur, Georgia	A	0–12	.....	16.2 (0.132)	.....	.....	14.0 (0.100)	.....	.....	.....
	B_1	12–36	.....	0.00 (0.000)	.....	.....	3.6 (0.026)	.....	.....	.....
Cecil, South Carolina	A_1	0–2	.....	8.4 (0.069)	.....	.....	.....	.....	.....	14.2 (0.099)
	A_2	2–8	.....	.....	0.0 (0.000)	6.7 (0.067)	.....	.....	.....	12.3 (0.086)
Georgeville, North Carolina	A_2	½–5	.....	0.0 (0.000)	.....	.....	.....	9.8 (0.092)	12.2 (0.097)	.....
	B_1	8–28	.....	0.0 (0.000)	.....	.....	.....	0.0 (0.000)	.....	.....
Norfolk, North Carolina	A_1	0–2	5.7 (0.038)	.....	.....	.....	.....	.....	.....	.....
	A_2	2–13	0.0 (0.000)	.....	.....	.....	0.0 (0.000)	.....	0.0 (0.000)	.....
Indoleacetic acid, 50 micrograms/liter	.....	.....	22.5	18.4	25.4	15.0	21.1	16.0	18.7	21.5

ments mentioned indicate that the pH of these soils should not be destructive to auxin, as a more acid soil would be expected to be.

Recently LAUDE (**8**) has shown that a 10^{-9} molar solution of indoleacetic acid, when applied to tomato plants grown in washed quartz sand and with added inorganic nutrients, increased their dry weight 12.5 per cent more than control plants receiving the inorganic nutrients alone. Again the concentration of auxin in the fertile soils seems to approach this optimum.

While there are several claims that indoleacetic acid and other auxins added to soil stimulate growth (**3, 18, 9, 14, 5, 10, 20**), there are nearly as many claims that this is not so (**11, 15, 7, 4**). The present data make no case for the desirability of adding auxin to soil as a means of stimulating plant growth, but they indicate that fertile soils tend to contain more auxin than less fertile ones, and—except in the most fertile—surface soils more than the subsoils. This suggests that natural soil auxin may, under certain conditions, have some stimulative effect upon plant growth. Also it offers the possibility of an additional partial explanation for the infertility of subsoils so frequently noted.

Summary

1. A proximate method is described for the extraction and assay of auxin in soils. One hundred gm. of soil is shaken at intervals for 48 hours with sufficient limewater to keep the pH between 7 and 8 in a volume of 400 cc. The filtered extract is then concentrated and auxin measured by the standard *Avena* test.

2. Auxin determinations were made on the two upper horizons of eleven virgin soils representative of four of the great soil groups. In very fertile midwestern surface soils and subsoils auxin was found to the extent of about 0.175 micrograms per kilogram of soil (indoleacetic acid equivalents). In less fertile soils considerably less auxin was found in the surface horizons and little if any in the second horizon.

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EFFECT OF PHENYLACETIC ACID AND NAPHTHALENE ACETAMIDE ON TOMATO PLANTS GROWN IN SOIL[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 539

MERLE EDISON HAMNER

Introduction

There are many reports on the growth-promoting effects of some of the growth-regulating substances when applied to plants growing in soil. In relatively few of these do the results presented lend themselves to an actual statistical examination. Often no detailed data of any kind are given. Recently LAUDE (2) presented data which showed that, when grown in sand culture and under certain conditions of potash supply, there may be an increased growth of red kidney bean plants when very small quantities of indoleacetic acid are supplied in the nutrient medium. When under similar conditions small quantities of naphthalene acetamide were supplied instead of indoleacetic acid, the growth of the tops was significantly reduced, but the dry weight of the roots was increased. SWARTZ (4) also found that in sand culture, marigold and cosmos showed either a decrease or no significant increase when small amounts of vitamin B_1, nicotinic acid, naphthaleneacetic acid, or combinations of these substances were added to the nutrient solution. C. L. HAMNER (1), using sand culture and either complete nutrient solution or one low in phosphate content, also found increased root growth and decreased top growth of bean plants when naphthalene acetamide was supplied in the culture solution. There was some evidence of increase in top growth of tomato plants when phenylacetic acid was present in the culture solution in a concentration of 10^{-8}, but such evidence was not conclusive. The higher concentrations under similar conditions resulted in lesser growth. Thus it appears reasonably certain that when certain of the growth-regulating substances are supplied in relatively high dilution in the nutrient solution to sand cultures, there may be an increase in growth over the controls.

Recently STEWART and ANDERSON (3) have shown that it is possible to extract auxins from certain native soils in sufficient quantity to be detectable by the *Avena* test, and at a concentration such that they might possibly affect the quantity and rate of growth of plants grown in such soils. In view of the data just mentioned, and those in many other reports, it was decided to test the possible effects

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of phenylacetic acid and naphthalene acetamide on plants grown in a native garden soil and also in another soil to which sand and calcium carbonate were added.

Experimentation

Two soils were selected. Soil I was a fertile garden soil from Lake Geneva, Wisconsin. Abundant crops of flowers and vegetables had been grown on it for several successive years. It was high in thoroughly decayed native organic matter, since it came from low land near the lake shore. Upon testing, it was found to have a pH of 7.3. Soil II was basically a clay loam from near Chicago, and on it also crops of vegetables had been grown for several years. It was dark in color and fairly high in organic matter. After drying, this soil was crushed into fine pieces and then mixed with quartz sand on the basis of one part sand to five parts soil by volume. To this combination finely powdered calcium carbonate was added at the rate of 5 pounds of carbonate to each $1\frac{1}{2}$ cubic yards of soil. This combination was very thoroughly mixed. Upon testing it had a pH of 7.8.

Several hundred glazed crocks of $1\frac{1}{2}$-liter capacity with side drainage were filled with these respective soils. They were arranged in rows of eight pots each. The pots containing one type of soil were distributed on two benches and those of the other type on two other benches, in a large greenhouse. The total number of crocks used was divided into twelve lots. Six lots contained soil I and six, soil II. Each lot consisted of ten rows of eight crocks. Five rows were used for the four concentrations of phenylacetic acid and its control and five for the naphthalene acetamide. The rows in each lot were randomized by successive drawings by chance of the numbers 1 to 5, to conform with the concentrations of growth-regulating substances used, and the rows were labeled accordingly, the rows of any specific concentration of the two substances standing side by side. Each pot was thoroughly watered with distilled water and allowed to stand several days before anything was planted in it.

On October 2, 1941, Bonny Best tomato seeds were planted in previously unused quartz sand and then lightly watered with distilled water. Thirteen days later, when the seedlings were about $1\frac{1}{2}$ inches high, the cotyledons spread apart but the epicotyl scarcely showing signs of elongation, uniform plants were selected and two were planted in each of the glazed crocks already filled with the respective soils. Later they were thinned to one plant per crock. The control plants grew well for the season of the year and were just coming into flower when harvested.

To avoid the use of tap water or distilled water in watering the plants, or as a medium in which to supply the growth substances, a complete nutrient solution—consisting of 0.0045 mol. $MgSO_4$, 0.0045 mol. KH_2PO_4, 0.0060 mol. $Ca(NO_3)_2$, together with the minor elements—was made up. This was then diluted to one part in twenty with distilled water. Whenever the plants were watered or the

growth substances were supplied, 100 cc. of the diluted medium with or without the growth substances added was used per crock.

The two growth substances tested were phenylacetic acid (Eastman) and naphthalene acetamide (American Chemical Paint Company). A concentrated solution was made up by dissolving the crystals in 95 per cent ethyl alcohol, and

TABLE 1

DRY WEIGHTS IN GRAMS OF THE SEVERAL RANDOM SAMPLES AT TIME OF HARVESTING

Lot	Substance and concentration									
	0		10^{-10}		10^{-8}		10^{-6}		10^{-4}	
	Control	Control	N*	P*	N	P	N	P	N	P
	Soil I									
III	8.9	10.2	7.3	8.3	7.6	8.2	7.5	8.1	2.9†	6.7
IV	9.4	9.7	8.9	8.5	8.0	8.1	7.9	8.9	3.3†	6.8
V	8.9	9.2	7.7	8.4	7.9	8.5	8.2	8.2	3.2†	7.0
X	8.7	8.4	8.1	8.1	7.9	8.9	8.0	8.4	3.2†	6.7
XI	9.3	9.0	8.7	8.4	7.5	8.0	7.9	8.2	2.6†	6.9
XII	9.5	10.4	8.1	8.5	9.2	8.9	8.7	8.6	2.6†	8.1
Total	54.7	56.9	48.8	50.2	48.1	50.6	48.2	50.4	17.8†	42.2
	Soil II									
I	7.3	7.6	7.1	7.2	7.1	6.7	7.1	6.9	0.3	5.7
II	8.3	7.9	7.1	7.5	6.2	7.2	5.8	5.7	0.4	6.0
VI	7.7	7.2	7.6	7.0	6.5	7.2	6.6	6.9	0.3	5.6
VII	8.0	8.5	6.8	7.3	7.3	7.5	7.6	6.3	0.3	6.0
VIII	8.1	9.4	8.1	8.4	8.5	7.0	7.9	7.8	0.4	7.1
IX	9.5	8.5	8.0	8.0	7.9	7.9	8.3	8.9	0.7	7.6
Total	48.9	49.1	44.7	45.4	43.5	43.5	43.3	42.5	2.4	38.0

* N, naphthalene acetamide; P, phenylacetic acid.

† When watered on October 22, only ⅛ of the usual amount of naphthalene acetamide was supplied.

then—just before use—the proper amount was added to the diluted nutrient solution to make up the desired concentrations of the respective substances. The concentrations used were 10^{-4}, 10^{-6}, 10^{-8}, 10^{-10}, and 0.

The crocks containing soil I were watered with nutrient containing the respective growth substances October 22, November 9, 16, 24, 30, December 7. Soil II was watered October 30, November 9, 16, 24, 30, and December 7. On December 15 the plants constituting any given sample were carefully cut off at the cotyledonary node near the surface of the soil, the plants placed in a paper sack and

dried rapidly to brittleness. Later each sample was again dried to constant weight at 100° C.

Table 1 is a record of all the samples. The results showed considerable variation within any given treatment, depending upon their particular location on the greenhouse benches. As a whole, the plants in soil I made more growth and had greater

TABLE 2

AUXIN CONTENT OF SOILS, EXPRESSED AS MICROGRAMS OF INDOLEACETIC-ACID EQUIVALENTS PER KILOGRAM OF AIR-DRY SOIL

Lot	Substance and concentration									
	0		10^{-10}		10^{-8}		10^{-6}		10^{-4}	
	Control	Control	N*	P*	N	P	N	P	N	P
	Soil I									
I	0.000	0.000	0.035	0.000	0.000	0.000	0.000	0.000	0.031†	0.000
II	0.029	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.020†	0.000
VI	0.042	0.000	0.000	0.039	0.063	0.000	0.000	0.000	0.140†	.....
VII	0.035	0.128	0.000	0.126	.....	0.000	0.051	0.000	0.093†	0.000
VIII	0.082	0.046	0.000	0.158	0.090	0.034	0.000	0.000	0.031†	.....
IX	0.033	0.000	0.083	0.025	0.057	0.000	0.020	0.014	0.000†	.....
Average	0.032	0.029	0.032	0.058	0.030	0.006	0.017	0.002	0.045†	0.000
	Soil II									
III	0.000	0.038	0.000	0.114	0.000	0.000	0.000	0.155	0.000	0.000
IV	0.000	0.125	.....	0.000	.....	0.000	0.000	0.000	0.000	0.000
V	0.000	0.131	.....	0.034	0.000	0.000	0.000	0.000	0.000	0.075
X	0.122	0.129	0.000	0.038	0.000	0.150	0.142	0.000	0.000	0.127
XI	0.024	0.000	0.000	0.000	0.118	0.104	0.000	0.000	0.076	0.000
XII	0.000	0.170	0.000	0.102	0.000	0.000	0.022	0.147	0.023	0.115
Average	0.024	0.099	0.000	0.048	0.024	0.042	0.027	0.050	0.014	0.053

* N, naphthalene acetamide; P, phenylacetic acid.

† When watered on October 22, only $\frac{1}{8}$ of the usual amount of naphthalene acetamide was supplied.

dry weight than those in soil II, but with respect to their responses to the growth-regulating substances the trends in the two soils were similar. This trend clearly shows progressively less total dry weight with increasing concentration of the growth substance supplied, with a very abrupt decrease between 10^{-6} and 10^{-4}. This decrease is much more marked in the case of the plants treated with naphthalene acetamide. None of the concentrations resulted in any increase in dry weight. Except for the fact that none of the results showed an increase, the results in gen-

eral closely approximated those obtained when quartz sand and a nutrient solution were used instead of soil. No measurements were made on root development.

After all the plants had been harvested, comparable samples of each random lot of soil were made up by combining aliquots from each of the pots in any given row. These samples were sent to Dr. WILLIAM S. STEWART at Beltsville, Maryland, who tested them according to his method of determining the amount of auxin in soils. The results of these determinations are given in table 2, the random samples being listed in the same sequence as they occur in table 1. At present no deductions are drawn from the data, other than the fact that the two growth-regulating substances seem to have had little or no effect, either in increasing or in decreasing the amounts of detectable auxin in the two soils. The figures are presented chiefly to show the wide range of variation in the results obtained; such variation may be partly inherent in the method of testing (3).

As stated, the results from this experiment are similar to those obtained when quartz sand and various nutrient solutions were used as a medium for growing the plants, instead of soil, except that in no instance were there increases in growth over that of the controls. Even the most dilute concentration, 10^{-10}, resulted in less growth. There are no clear, direct indications that the physical texture, the soluble salt content, or the quantity or type of organic matter present in these soils directly influence the general effects of these two growth substances when supplied to tomato plants. The results do not at present support the rather generalized claim that the growth of plants is greatly increased when small quantities of either of these substances is added to the soil.

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RIBOFLAVIN IN ISOLATED ROOTS[1]

JAMES BONNER

Riboflavin is not known to be an indispensable growth supplement for any isolated root whose continued cultivation *in vitro* has yet been reported. Although it is possible that roots requiring supplements of this substance may be found, still it is evident that an outside source of riboflavin is not required by roots of tomato, alfalfa, clover, and others which are known to require supplements of thiamin, nicotinic acid, and/or vitamin B_6. The data presented here suggest that riboflavin is synthesized by certain isolated roots.

Isolated roots were cultured according to methods reported earlier (**1**). The culture medium contained, per liter of redistilled water, 236 mg. $Ca(NO_3)_2 \cdot 4H_2O$, 36 mg. $MgSO_4 \cdot 7H_2O$, 81 mg. KNO_3, 65 mg. KCl, 20 mg. KH_2PO_4, 1.5 mg. ferric tartrate, and 20 gm. sucrose. Supplements of thiamin and vitamin B_6 were added in the concentration of 0.1 mg. per liter of nutrient and nicotinic acid at the rate of 0.5 mg. per liter of nutrient. Supplements of thiamin and nicotinic acid only were used for roots of alfalfa and clover; supplements of thiamin, vitamin B_6, and nicotinic acid were used for all the other species.

All roots were subcultured at weekly intervals by removal of a 1-cm. tip (from the principal axis of the root) to fresh medium. The roots of alfalfa, clover, *Datura*, and sunflower had been in culture for more than 1 year when the experiments were initiated.

Assays for riboflavin were done by the microbiological method of SNELL and STRONG (**2**), using *Lactobacillus casei* as the test organism. Growth was allowed to take place for 24 hours at 37° C. and was then measured in a Fisher electrophotometer. In each experiment a linear relationship between growth of the organism as measured in this way and riboflavin supplement obtained over the range 0.00 to 0.10 γ (micrograms) of riboflavin. Duplicate determinations on the same sample differed by less than 5 per cent.

Extraction of riboflavin was carried out by autoclaving two roots in 5 cc. of water at a pressure of 15 lb. for 15 minutes. The water was then decanted, made up to 5 cc., 5 cc. of basal medium added, and the riboflavin determination made on the sample. Where 1-cm. root tips were assayed the extraction was done similarly, except that ten (five each for sunflower and *Datura*) tips were used per 5 cc. of water.

[1] Report of work done with the co-operation of the Work Projects Administration, O.P. no. 165-1-07-172. This work was made possible in part by the support of Merck and Company.

Table 1 gives the results of a preliminary experiment which shows that the apparent riboflavin content of isolated tomato roots is independent of the amount of root extract used as a supplement for *Lactobacillus* in the riboflavin assay. The

TABLE 1

DETERMINATION OF RIBOFLAVIN IN DIFFERENT AMOUNTS OF EXTRACT OF ISOLATED TOMATO ROOTS. ALL PHOTOMETER READINGS (ARBITRARY UNITS) HAVE THE BLANK SUBTRACTED. ALL FIGURES ARE MEANS OF DUPLICATE DETERMINATIONS

Supplement	Photometer reading	Supplement	Photometer reading	Calculated amount of B_2 (micrograms)	Recovery of added B_2	
					Amount	Percentage
None	0.0	Extract of 1 root	7.6	0.0196/root	.....	.....
0.025 γ B_2	10.4	Extract of 2 roots	15.8	0.0203/root	.....	.....
0.05 γ B_2	18.6	Extract of 4 roots	30.5	0.0197/root	.....	.....
0.075 γ B_2	28.8	Extract of 1 root +0.05 γ B_2	26.1	0.0673	0.048	96
0.10 γ B_2	38.6	Extract of 2 roots +0.05 γ B_2	35.3	0.0910	0.050	100
		Extract of 4 roots +0.05 γ B_2	50.6	0.130	0.051	102

TABLE 2

CONCENTRATION OF RIBOFLAVIN IN ISOLATED TOMATO ROOTS OF VARIOUS AGES. ASSAYS ON INITIAL ROOT TIPS ALL MADE ON INDIVIDUAL LOTS OF 10 ROOTS. ASSAYS ON CULTURED ROOTS MADE ON LOTS OF 2 ROOTS

Initial root tips (1 cm. long)			Cultured roots (without tip)		
Experiment no.	Weeks in culture	γ B_2/root tip	Experiment no.	Weeks in culture	γ B_2/root
2	0	0.0024	2	8	0.015
		.0024			.016
		.0018			.018
3	0	.0014			0.026
		.0019			
		.0016			Av. = 0.019
		.0028			
		.0013	3	13	0.016
		0.0014			.014
					0.018
		Av. = 0.0019			
					Av. = 0.016
					0.020
			4	24	.020
					0.020
					Av. = 0.020

data also show that added riboflavin may be quantitatively determined in the presence of the extract of isolated tomato roots. It does not appear, therefore, that substances present in such roots interfere in any way with the results of the assay.

Experimental results

A clone of isolated tomato roots was established by the propagation of branch tips of a root derived from one individual seedling (San Jose Canner, of the California Packing Corporation) root tip. Seedling tomato root tips 1 cm. long and

TABLE 3

CONCENTRATION OF RIBOFLAVIN IN ISOLATED ROOTS OF CLOVER AND ALFALFA. INITIAL ROOT TIP ASSAYS BASED ON 10 TIP SAMPLES. ASSAYS OF CULTURED ROOTS BASED ON 2 ROOT SAMPLES

ALFALFA		CLOVER	
SOURCE OF ROOT	γ B_2/ROOT	SOURCE OF ROOT	γ B_2/ROOT
Initial root tips	0.0008	Initial root tips	0.0003
	.0017		.0003
	.0011		.0014
	.0008		.0008
	.0011		0.0008
	0.0019		
	Av. 0.0012		Av. 0.0007
In culture 68 weeks		In culture 68 weeks	
	0.009		0.021
	.015		.018
	.014		.029
	0.009		.015
			.018
	Av. 0.012		.012
			0.014
			Av. 0.018
In culture 70 weeks		In culture 70 weeks	
	0.014		0.024
	.014		.020
	0.018		.024
			0.021
	Av. 0.015		Av. 0.022
In culture 75 weeks		In culture 75 weeks	
	0.014		0.011
	.016		.011
	.011		.009
	0.011		0.011
	Av. 0.013		Av. 0.0105

similar to that from which the clone was derived were assayed for total riboflavin content. A series of determinations are given in the first column of table 2. After the clone had been propagated through eight successive weekly transfers, approximately 125 roots—averaging 50 mm. in length—were available. These roots were transferred by removal of the apical 10 mm. to fresh medium. Eight of the remain-

ing bases were then assayed for total riboflavin content. The procedure was repeated after the thirteenth and the twenty-fourth week in culture. These results are given in the second column of table 2. It is evident that the base (without the apical 10 mm.) of one cultured root contains roughly ten times as much riboflavin as does a root tip similar to that from which the entire clone was derived. At the end of the eighth week in culture, therefore, the entire stock of this clone would

TABLE 4

CONCENTRATION OF RIBOFLAVIN IN ISOLATED ROOTS OF DATURA AND SUNFLOWER. INITIAL ROOT TIP ASSAYS BASED ON 5 TIP SAMPLES. ASSAYS OF CULTURED ROOTS BASED ON 2 ROOT SAMPLES

DATURA		SUNFLOWER	
SOURCE OF ROOT	γ B_2/ROOT	SOURCE OF ROOT	γ B_2/ROOT
Initial root tips	0.0025	Initial root tips	0.0022
	.0025		.0034
	.0023		.0028
	0.0032		.0038
			.0044
	Av. 0.0026		0.0038
			Av. 0.0034
In culture 63 weeks		In culture 70 weeks	
	0.0155		0.013
	0.0155		.010
			0.008
	Av. 0.0155		
			Av. 0.010
In culture 68 weeks		In culture 75 weeks	
	0.014		0.021
	.018		.022
	.016		0.022
	0.014		
			Av. 0.022
	Av. 0.0155		

appear to have contained roughly 125 × 10, or over 1000 times as much riboflavin as did the original root tip. This would seem to indicate a riboflavin synthesis by the particular clone of isolated tomato roots in question.

Experiments generally similar to that just described were carried out on other species of isolated roots. Table 3 gives data concerning alfalfa and white clover. The two stocks of isolated roots used were not clones, but each had been increased in number by the propagation of branch root tips. Seedling root tips for riboflavin assay were obtained from the same stock of seeds as was originally used for establishing the isolated root stocks. The bases (after removal of 10-mm. apical growing points) were first assayed after sixty-eight successive weekly transfers. In the

sixty-eighth transfer the alfalfa roots had grown an average of 137 mm. and the clover roots an average of 106 mm. It is evident from table 3 that the bases (without 1-cm. apical tips) of both species contain more riboflavin than 1-cm. seedling root tips similar to those from which the isolated roots derived. The data for the seventieth and seventy-fifth weeks of culture support this view.[2]

Table 4 gives data similar to that of table 3 but pertaining to isolated roots of *Datura* and sunflower. The *Datura* roots represented a clone while the sunflower roots came originally from thirty individual seedling root tips, although the stock had been multiplied by the cultivation of branch root tips. The *Datura* roots had grown an average of 78 mm. during the sixty-third week and the sunflower roots an average of 52.5 mm. during the seventieth week when the first assays of table 4 were made. In each case seedling root tips were obtained from the same stocks of seeds as those used for obtaining the isolated roots. Although the data of table 4 are not extensive, they do indicate that the bases of cultured *Datura* and sunflower roots contain more riboflavin than seedling root tips similar to those from which the isolated roots derived.

Summary

1. The riboflavin content of 1-cm. root tips from aseptically germinated seedlings of five different species has been determined. In each case a similar root tip or tips was cultured *in vitro*.

2. Riboflavin determinations were done on isolated roots from the stocks thus established. The roots used for assay were bases from which 1-cm. apical tips had been removed and represented samples of the root tissue produced during each weekly culture period. The number of such bases which arose, during culture, from one initial seedling root tip was large; in the case of *Datura*, for example, over 1000 were actually produced during 63 weeks of culture, and more could have been produced had every branch tip been cultured. With every species, each of these bases contained markedly more riboflavin than the initial root tip from which the root or clone was derived. This evidence suggests that in all the species of isolated roots investigated, a synthesis of riboflavin took place during culture.

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[2] No explanation can be given for the apparent low riboflavin content of the 75-weeks clover roots. The roots were in good condition and had grown an average of 107 mm.

EFFECT OF MINERAL NUTRITION ON THE ASCORBIC-ACID CONTENT OF THE TOMATO

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(WITH THIRTEEN FIGURES)

Introduction

A review of the literature concerning the nutritive value of the tomato fruit (3) has indicated that tomatoes may contribute significant amounts of ascorbic acid and provitamin A to the human diet, and that the relative amount of these constituents may vary considerably. It is known that these variations may be partially correlated with differences in the hereditary constitution of the plant and with environmental relations during the growing season. This series of investigations has been designed to measure the influence of environmental factors on the nutritive value of the tomato, and no attempt is made to measure genetic influences.

The environmental factors may be arbitrarily classified as: (1) factors of the root environment which are influenced largely by soil conditions, that is, supply of nutrient elements to the roots, temperature, aeration, moisture supply, etc.; (2) factors of the top environment which are influenced largely by climatic conditions, that is, temperature, light intensity, light quality, length of photoperiod, relative humidity, etc.

In this paper, ascorbic acid is the nutritive quality considered. The investigations have emphasized variations in the growth and development of the tomato plant which result from differences in the supply of macronutrient elements in sand culture. Results of a few experiments which measure the influence of factors other than mineral nutrition are included. Experiments concerning the influence of microelement nutrition—as well as the effect of other individual environmental factors on nutritive value—are in progress, and constituents other than ascorbic acid are being considered.

Four separate experiments have been carried out, using the Bonny Best variety of tomato as the experimental material. The first two are concerned with the influence of wide variations in the supply of macronutrient elements to plants growing in sand culture upon the growth, fruit production, and ascorbic-acid content of the fruit. The third and fourth deal with the ascorbic-acid content of fruit produced by plants under similar conditions of nutrient supply but subjected to variations in other environmental conditions.

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Investigation

Experiment I

Methods.—The method of preparing nutrient solutions was adapted from the technique described by C. L. Hamner (2). Six stock solutions were prepared with nine salts, using concentrations as given in table 1. In so far as the major nutrient ions are concerned, each cation stock solution contains only one cation but all three anions. The concentration of anions in the cation stock solutions is constant. Conversely, each anion stock solution contains one anion but all three cations in comparable concentrations. The stock solutions were combined on the basis of

TABLE 1

Ionic concentrations of nutrient stock solutions in milliequivalents per liter*

Salt	Ionic concentrations of individual stock solutions					
	K	Ca	Mg	NO_3	H_2PO_4	SO_4
KNO_3	12.0			4.5		
KH_2PO_4	4.5				4.5	
K_2SO_4	9.0					4.5
$Ca(NO_3)_2$		12.0		12.0		
$Ca(H_2PO_4)_2$		4.5			12.0	
$CaSO_4$		9.0				12.0
$Mg(NO_3)_2$			12.0	9.0		
$MgHPO_4$			4.5†		9.0	
$MgSO_4$			9.0			9.0

* To each stock solution was added: 0.5 p.p.m. B as HBO_3, 0.5 p.p.m. Mn as $MnCl_2$, 0.05 p.p.m. Zn as $ZnSO_4$, 0.02 p.p.m. Cu as $CuSO_4$, and 5.0 p.p.m. Fe as $FeC_6H_5O_7$.

† H_3PO_4 was added to this solution to raise the concentration of the H_2PO_4 ion to the required strength.

ninths to secure a range of twenty-eight solutions of varying cation concentrations and twenty-eight solutions of varying anion concentrations (fig. 1). Thus the apical position of the cation triangle lacks two cations; each side of the triangle lacks one cation; and the center portion of the triangle is supplied with all three cations in varying proportions. All treatments in the cation triangle are supplied with anions in equal concentrations.

On August 15, 1940, several thousand commercial seeds were planted in pure quartz sand and watered with a complete nutrient solution (position 13, fig. 1) which had been diluted with distilled water in the ratio of 1:2. The seeds were planted in small crocks, fifteen per crock, and after germination the seedlings were thinned out, leaving two uniform seedlings in each pot. The plants were watered with the complete nutrient solution once each week and at other times during the week were supplied with small amounts of distilled water.

On September 12 the plants were 28 days old and approximately 4 inches high. At this time the seedlings were selected for uniformity and were transplanted

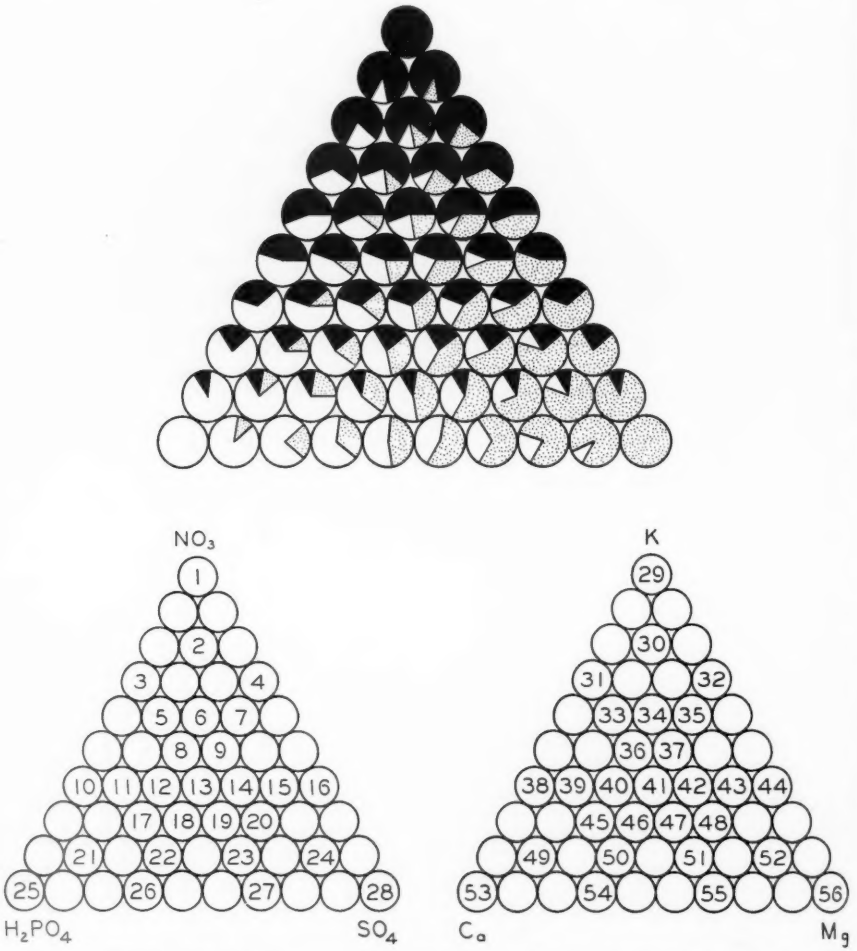

FIG. 1.—Upper triangle is a diagrammatic representation of relative proportions of stock solutions used to produce 55 possible combinations. Left, below: 28 combinations of anion stock solutions used in experiment I. Right, below: 28 combinations of cation stock solutions used in experiment I.

into 2-gallon glazed crocks containing pure quartz sand. Crocks totaling 1120 were used and each crock contained two seedlings. The design provided for fifty-six nutrient treatments with twenty crocks (forty plants) per treatment. The

treatments were randomized on the greenhouse bench to minimize the influence of environmental variations on the results. The plants were watered twice weekly with nutrient solution and were flushed thoroughly once a week with distilled water, prior to application of the nutrient. At other times the plants were watered with distilled water as needed. The greenhouse temperature was maintained as nearly as possible at 70° F., and low atmospheric humidities were avoided by frequently sprinkling the walks and walls of the house with water. Extreme care was exercised to preclude the possibility of infections with parasites or insects of any sort. The plants were pruned weekly of all axillary growth, and as flowers formed in the various treatments, the clusters were tapped by hand to insure pollination. A smaller amount of distilled water was used in watering the crocks as the light intensity decreased in the fall, but the plants were watered with the nutrient solution twice each week, a sufficient quantity being used to provide an appreciable amount of drip from each pot.

A portion of the plant population was harvested at 61 days, when many were in bloom. Fresh and dry weight data were obtained, and details on the appearance recorded. The remaining portion of the population was maintained on the respective nutrient treatments until sufficient ripe fruits were analyzed for ascorbic acid.

The chemical method was used in the determinations of ascorbic acid with a modification described by MORELL (**6**). The aliquot, however, was titrated to an end point with standardized dye. A transverse section weighing approximately 20 gm. was taken from the equatorial portion of the fruit. Various methods of sampling have shown that this procedure gives an accurate estimate of the average value of the fruit.

EXPERIMENTATION AND RESULTS.—On October 15 all the plants were 61 days old and had been supplied with their respective nutrient solutions for 33 days. Eight crocks (sixteen plants) in each treatment were harvested, and, in addition, the tops of one plant from each of the twelve remaining crocks in each treatment were removed. The dry weights of these plants were determined (fig. 2). The total of twenty-eight plants is used as a criterion in the estimation of growth in a particular nutrient treatment.

It may be seen that the several nutrient solutions employed have resulted in differing amounts of growth as measured by accumulation of dry weight. It therefore seems apparent that the variations in the nutrient combinations were of sufficient magnitude to affect the behavior of the plants, even at this early date. This conclusion is supported by the appearance of the plants at this time. Certain symptoms which developed on plants in various treatments could be correlated with the absence of one or more ions in the nutrient medium. In general, a treat-

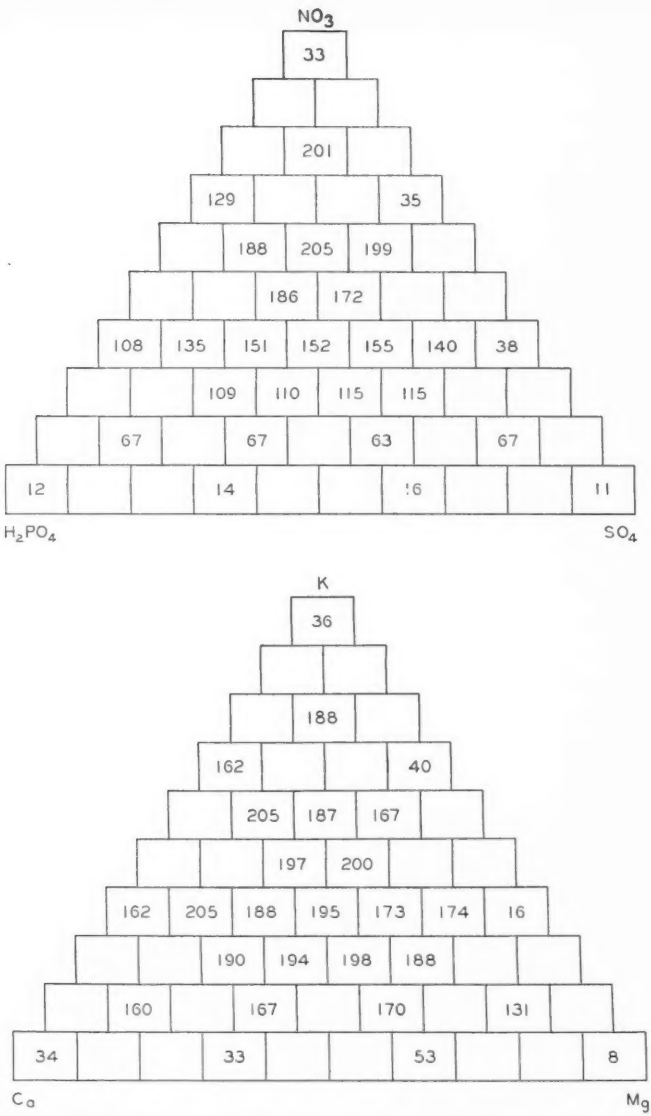

FIG. 2.*—Total dry weight in grams of the 28 vines of each treatment at first harvest of experiment I.

* In this and in all succeeding figures where data are presented, results are given in triangular positions corresponding to equivalent positions in figure 1.

ment deficient in two ions resulted in intermingling of symptoms characteristic of the respective single ion deficiencies and will not be discussed separately.

Phosphate-deficient treatments produced spindly plants with purple stems, deep purple-green upper leaves, and lower leaves which were pale green with a bronze cast. The dry weights were significantly less than the dry weights of plants produced under more optimum conditions of anion nutrition. The best appearance and growth in the phosphate-deficient treatments occurred in position 16, where relatively low nitrate and high sulphate conditions in the nutrient medium were prevalent.

Nitrate-deficient treatments produced small plants with pale yellow upper leaves. The lower leaves in general were dead or completely lacking. These symptoms were less striking in treatments with higher concentrations of nitrate and were not apparent under optimum conditions. Deficient treatments produced very little dry weight. With increasing nitrogen supply, the accumulation of dry matter became progressively greater. In the center portion of the triangle there is little or no indication of a sulphate or phosphate interaction.

In the two treatments when lack of sulphate was not accompanied by phosphate or nitrate deficiencies, sturdy light green plants with thin yellowish stems and chlorotic lower leaves were produced. The dry-weight accumulation was approximately 60 per cent of that produced under most optimum conditions of anion supply. When this deficiency was accompanied by the absence of another anion, the symptoms which developed more closely approximated those characteristic of the latter.

Of the plants in the cation triangle, those receiving solutions deficient in potassium were the smallest. The lower leaves were yellow, and brown spots developed on some of the younger leaves, with burning at the tips and margins of older leaves. A tendency for the leaves to curl downward was characteristic, especially with those solutions high in calcium and low in magnesium. Dry-weight accumulation of these plants was considerably less than in treatments represented in the center of the triangle.

In general, the plants grown in solutions deficient in calcium were small, with brown terminal buds which were in some cases dead, and in most cases the axillary buds turned brown and failed to develop. There were wide variations apparent in these treatments, and some of the plants were four or five times as tall as others. They grew vigorously, had fully expanded green leaves, and exhibited none of the symptoms of injury to the terminal bud. Occasionally one of the large plants was found growing in the same pot with another large plant, but more often they were found beside small plants showing extreme calcium deficiency. This variability in response to a nutrient supply deficient in calcium has been observed in other experiments. In this experiment, plants which were supplied with solutions con-

taining only magnesium as the cation were the smallest, and death of the terminal bud was not observed. In calcium-deficient treatments the accumulation of dry weight was relatively low.

At this time, plants grown in magnesium-deficient solutions which contained both calcium and potassium were light green, and the lower leaves were chlorotic. The dry-weight accumulation of these plants was approximately 80 per cent of the maximum obtained.

In both the anion and cation triangles, the treatments represented in the center portions produced healthy plants with no symptoms of chlorosis or necrotic areas on the leaves. The only apparent differences at this time were variations in size corresponding to those recorded in the dry-weight data.

Subsequent to the initial harvest, the remaining twelve crocks per treatment containing one plant per crock were redistributed and randomized in the greenhouse with enough space between them to provide the best available lighting conditions. The plants were continued on their respective nutrient treatments, and fruit started to ripen during the early part of December. Each fruit was picked on the morning of the day that complete color change had occurred. Immediately after removal, the fruit was weighed, an ascorbic-acid determination made on a representative sample, and the rest of the fruit prepared for analyses of other constituents. Only occasionally were two fruits removed from a single plant, and usually the fruit used represented the first one ripened. All determinations were made on the day that the fruit was picked, and the results of 318 analyses are given in figure 3. Within the limits of precision of this experiment, there are no significant differences in the ascorbic-acid content of fruit produced by different treatments. It is significant, however, that the average ascorbic-acid content of all the fruits that were analyzed (13.3 mg. per 100 gm. fresh fruit) was approximately 50 per cent of the average values reported in the literature (3). These tomatoes were produced in the greenhouse in the fall of the year, when low light intensities were prevalent. The data of other experiments have a direct bearing on this point.

The plants were continued on their respective nutrient treatments until December 19, 1940. At this time all remaining plants were harvested, and measurements of dry weight of root systems, dry weight of vine, and fruit production were recorded. It was noted that the dry weights as well as the quantity of fruit produced by the plants subjected to the various treatments were affected by relatively small variations in composition of the nutrient solutions. Variations occurred as the result of differences represented in the central region of the triangles. The symptoms characteristic of deficiencies were also apparent in treatments containing small amounts of certain ions. Since the trends shown in data for these characters closely coincide with results obtained in experiment II, they are discussed later.

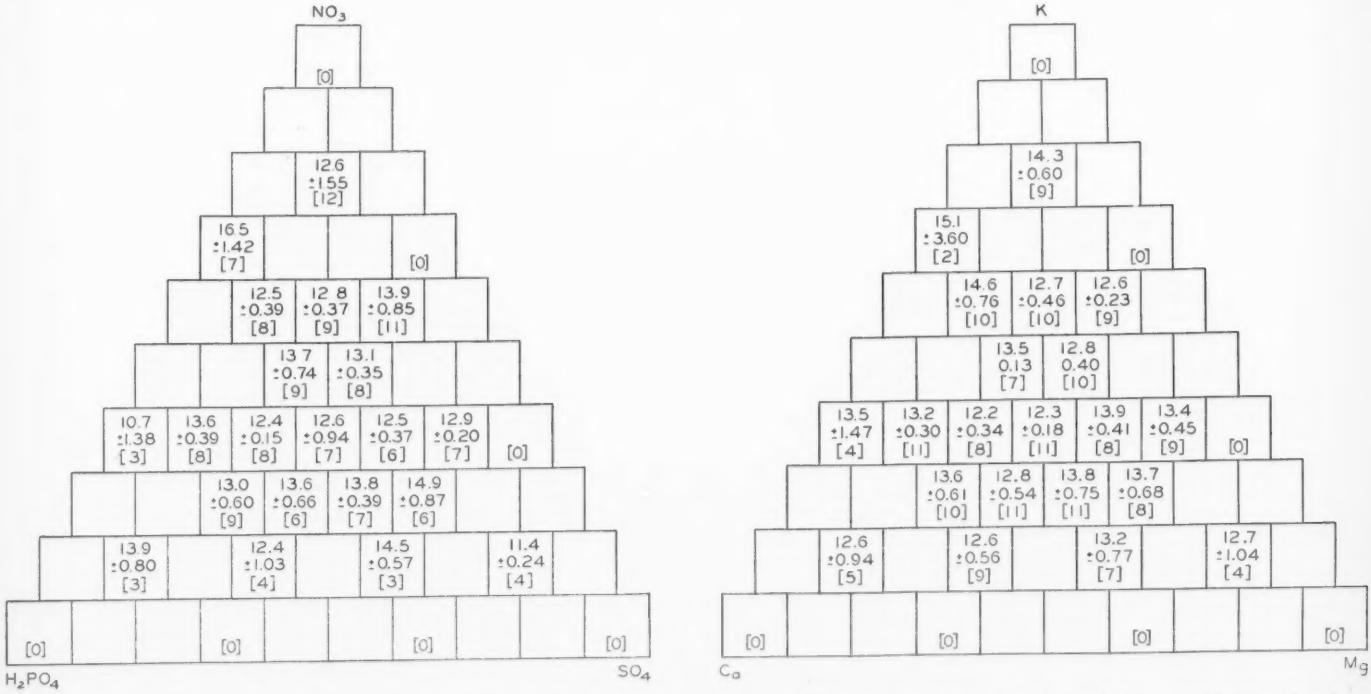

FIG. 3.—Ascorbic-acid values in experiment I (in mg. per 100 gm. fresh fruit), giving treatment means together with their standard errors. Number of analyses involved in each treatment recorded in brackets.

EXPERIMENT II

Since the results of experiment I indicated that the ascorbic-acid content of the tomato fruit is not affected to any appreciable extent by wide variations in the supply of macronutrient elements to the plant, and since all values were relatively low, it seemed desirable to repeat the experiment during the regular growing season and to grow the plants outside instead of in the greenhouse. Several modifications of experiment I were introduced, including the use of an inbred strain of tomatoes, the use of more nutrient combinations, the analysis of a greater number

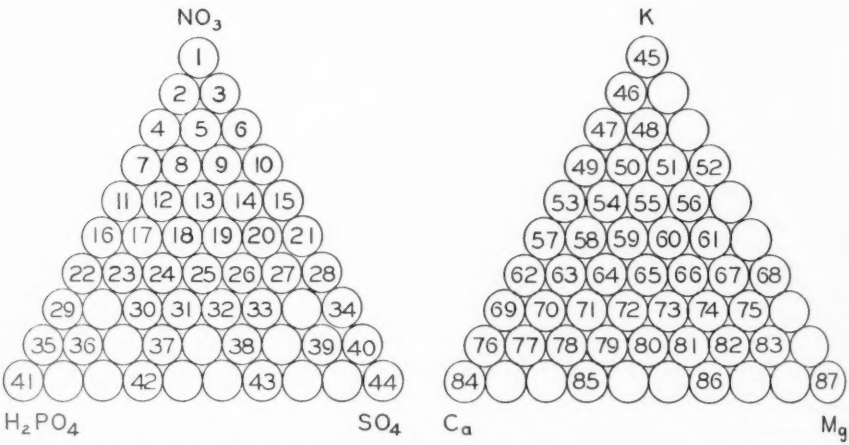

FIG. 4.—Left, the 44 combinations of anion stock solutions used in experiment II and right, the 43 cation combinations. Refer to figure 1 (top) for proportions of stock solutions in each combination.

of fruits for ascorbic acid, and a specific design for reduction and analysis of the data by statistical methods.

METHODS.—The inbred strain of the Bonny Best tomatoes used has been inbred for seven generations.[4] Seed was planted in the greenhouse on May 12, 1941, in 2-quart glazed crocks containing pure quartz sand. Two seeds were planted in each crock, and a complete nutrient solution (see treatment 25, fig. 4) was used during germination and seedling stage. On June 9, when the seedlings were 28 days old and approximately 3 inches tall, uniform seedlings were transplanted into 2-gallon glazed crocks containing pure quartz sand, one seedling per crock. The seedlings were watered in with distilled water and immediately supplied with their respective nutrient treatments. On July 1, all plants were placed outdoors, the crocks being supported on wooden blocks. The plants were trained upright,

[4] Seed supplied through the courtesy of Dr. LeRoy Powers, Senior Geneticist, U.S. Horticultural Field Station, Cheyenne, Wyoming.

and both crocks and plants were supported by stakes. All axillary growth was pruned off twice weekly, and the plants were harvested September 10.

The design of the experiment was that of a randomized block (1) with eighty-seven treatments and four replications. Each replication consisted of a three-plant row and was randomized by the use of TIPPETT's tables (8). Thus the mean of twelve plants was used as an estimation of the response to a given treatment, and a total population of 1044 plants was used. The data were reduced by means of the analysis of variance, and the *t* test (7) was used for determining whether particular differences were significant. Odds as great as or greater than 99:1 against the deviations noted, being due to the errors of random sampling, were accepted as statistically significant. Subsequent to the time the seedlings were transplanted, the same randomization and design was maintained both in the greenhouse and in the field.

Nutrient stock solutions were made up exactly as in experiment I, and the system of triangulation was the same. The number and position of the treatments, however, were modified (fig. 4). Thus, in this experiment, the nutrient stock solutions were combined on the basis of ninths to provide forty-four solutions of varying anion composition and forty-three of varying cation composition.

During the early growth stages, nutrient solution was supplied to the plants three times each week. As the summer progressed, nutrients were supplied every other day, and finally—when fruits were ripening—they were applied four times per week. The solutions were applied in quantities sufficient to insure an appreciable amount of drip, and each pot was watered with distilled water between nutrient applications.

During germination and the seedling stage, greenhouse temperatures were maintained at 85° F. in the daytime and 70° at night. Subsequent to transplanting, the temperatures in the greenhouse closely approximated those prevailing outside.

The mean daily temperature for the duration of the experiment was 67.7° F., with a mean maximum of 80.9° and a mean minimum of 55.3° F. The mean maximum temperatures in June, July, and August were 79.8°, 84.6°, and 79.3° F., respectively. Mean relative humidity from May 12 to September 10 was 45.2 per cent at 1:30 P.M. The average daily total of solar radiation for this period was 520.7 gram calories per square centimeter of horizontal surface.

EXPERIMENTATION AND RESULTS.—On July 23, all plants were 72 days old and had received their respective nutrient solutions for 46 days. At this time—and at weekly intervals thereafter—each plant was measured and the height in centimeters recorded. When the plants were 120 days old and had received their respective nutrient treatments for 94 days (September 9), they were harvested, the vines immediately weighed, and the number of elongated internodes counted.

The root systems were washed as free from sand as possible. Subsequent to this time, the vines and root systems were dried at 60° C. for 4 days in a forced-draft oven. The root systems were separated from all sand particles by pulverizing the dried material with adhering sand and separating these components by air pressure. Both the root systems and the vines of each plant were weighed and the dry weights recorded. These data were compiled, and the average length of internodes, the percentage dry matter of the vine, and the top-root ratio computed.

Fruit started to ripen in late July and early August. On July 25, data were recorded on the occurrence of blossom-end rot. These data, together with the results of mineral analyses on the fruit, are reported in a separate paper (**5**). All diseased fruits were discarded. Only healthy normal fruits were allowed to mature, and each fruit was picked on the morning of the day that complete color change had occurred. The entire population was examined daily until the end of the experiment. When picked, a fruit was immediately weighed, a portion analyzed for ascorbic acid, and the remaining portions prepared for analyses of other constituents. Only the first two fruits which ripened per plant were used for analytical procedures, but records were kept of the weight of every fruit as it was picked in order to compile data on total fruit production and mean fruit size. Upon completion of the experiment, all fruits were picked, counted, and weighed.

During the course of the experiment, careful notations were made on the gross appearance of the plants. The differences in the various treatments were similar to those recorded in experiment I and are not given in detail here. However, certain contrasts were obvious. The lack of uniformity in plants supplied with calcium-deficient nutrients noted in experiment I was not apparent in experiment II. Symptoms of calcium deficiency developed shortly after initiation of the treatments, and death of the terminal buds occurred within 32 days. Responses to low nitrate concentrations were more striking in the second experiment than in corresponding treatments of the first. Maximum growth occurred in comparable treatments in both experiments; but the plants were more sturdy, had thicker stems, produced more and larger fruit, and did not grow so tall in the second. The differences in environmental conditions of the two seasons represented should be kept in mind. It is, however, the similarities in response to treatment which should be emphasized. That the general trends of experiment I are duplicated in experiment II is interpreted as evidence that they represent actual responses on the part of the plant to variations in nutrient composition.

The results of the growth measurements are given in figures 5 and 6. The curves are presented largely for comparison and for an estimate of the relative rates of growth at different times. Data on growth and fruitfulness are given in figures 7 11.

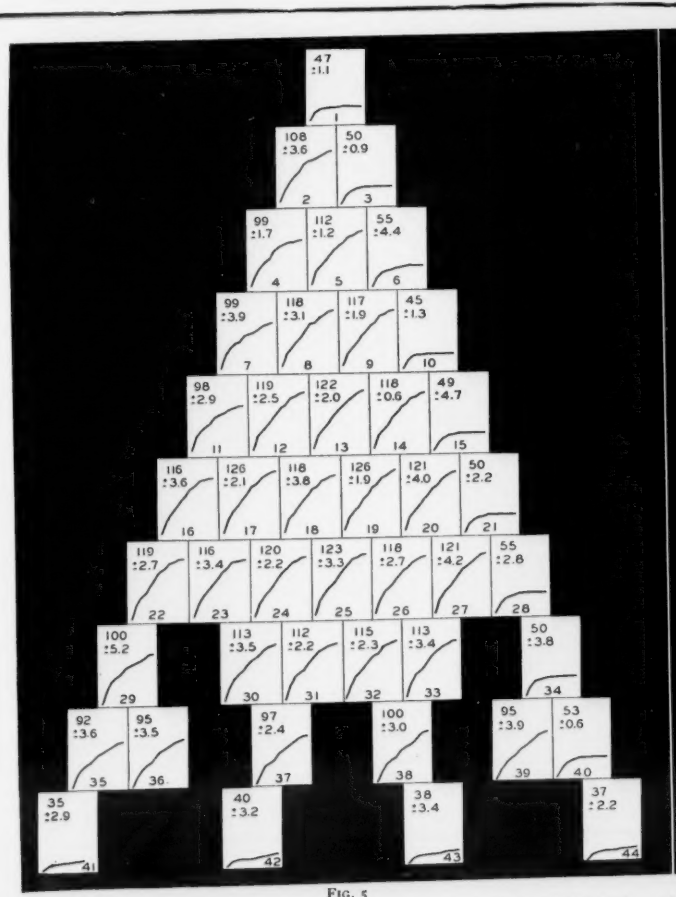

FIG. 5

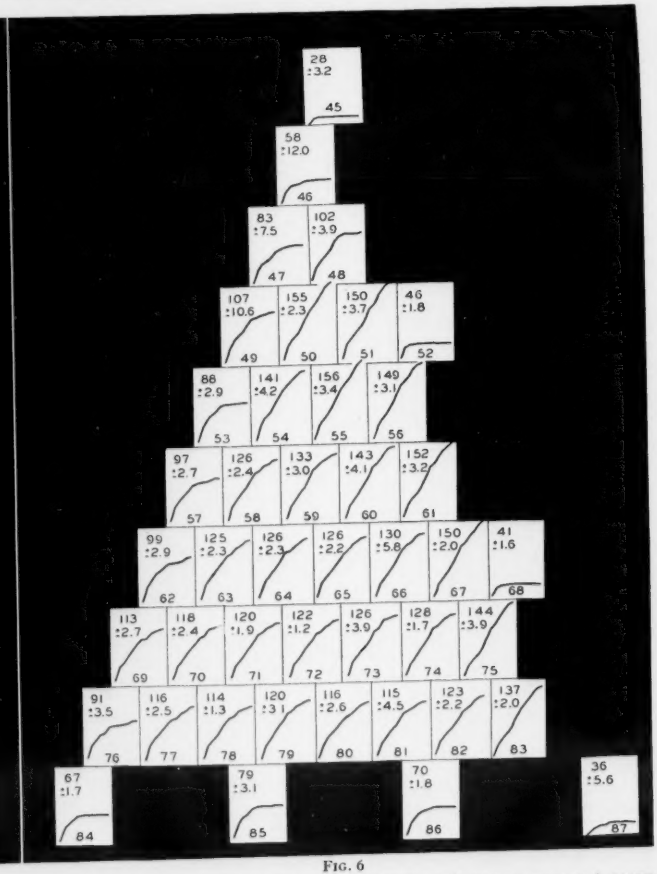

FIG. 6

FIG. 5.—Graph of average weekly height measurements of plants in anion treatments in experiment II. Data above each curve represent mean height in centimeters together with its standard error. Treatment numbers indicated below each curve. Fig. 6 (right), same as figure 5, except that cation treatments are presented.

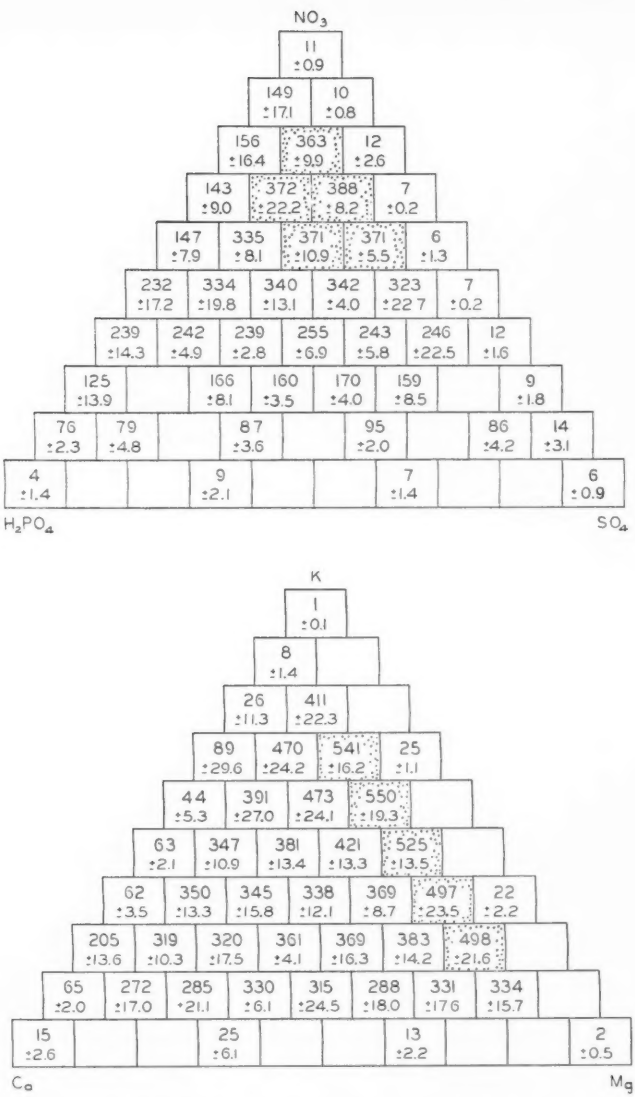

FIG. 7.*—Fresh weight of vine (in grams) for anion treatments (above) and cation treatments (below) of experiment II.

* In figs. 7–12, treatment means given together with their standard error. In any inter-treatment comparison, six degrees of freedom are available. When $t = 3.71$, $P = 0.01$.

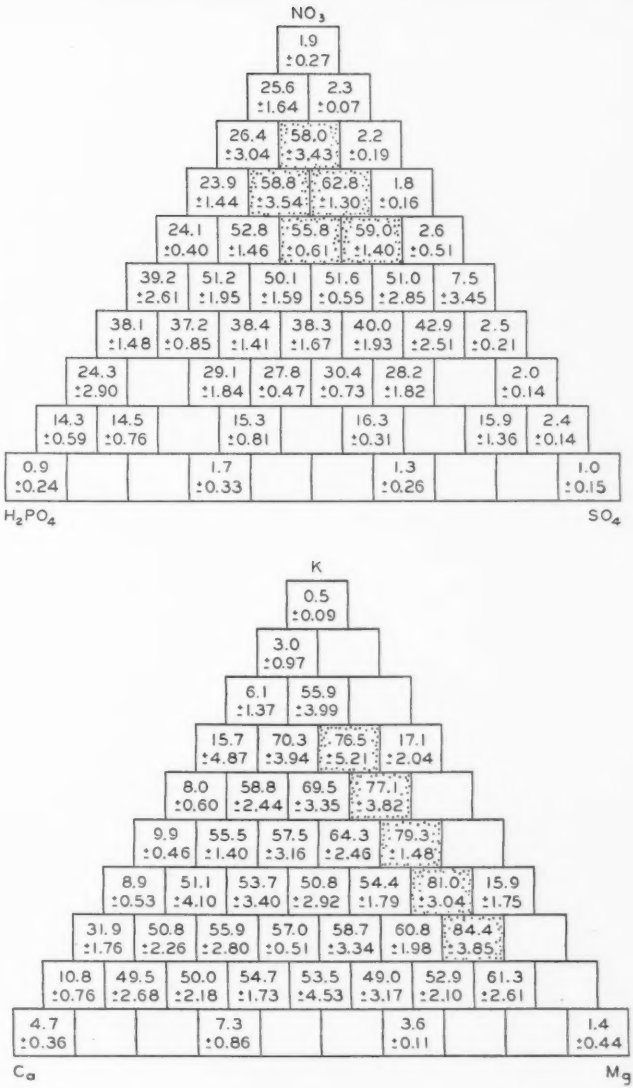

FIG. 8.—Dry weight of vine (in grams) for anion treatments (above) and cation treatments (below) of experiment II.

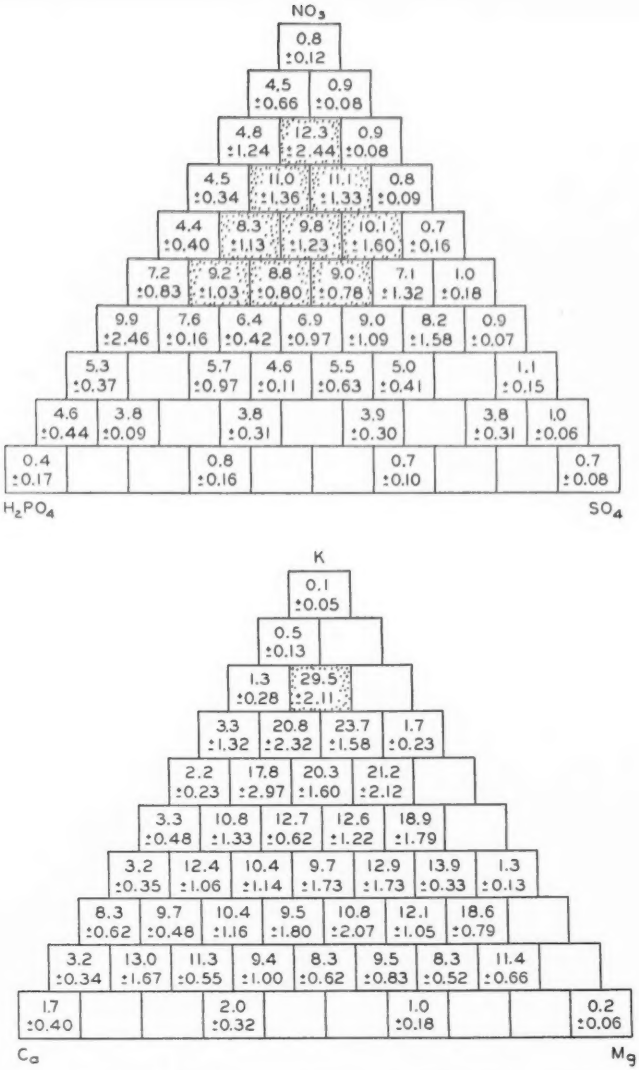

FIG. 9.—Dry weight of root system (in grams) for anion treatments (above) and cation treatments (below) of experiment II.

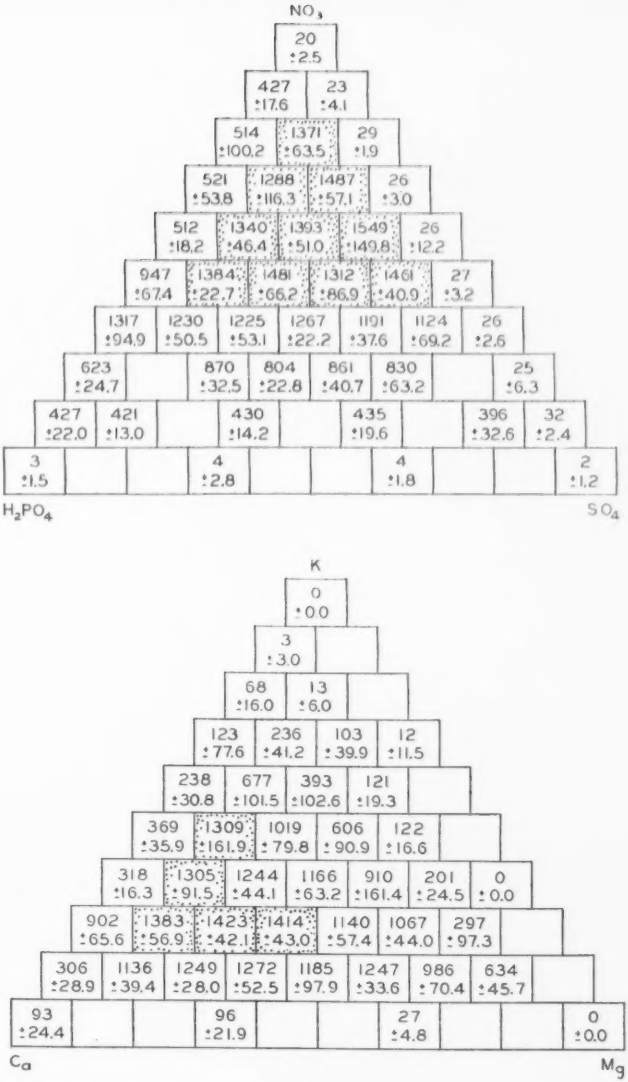

FIG. 10.—Total fresh weight of fruit per vine (grams) for anion treatments (above) and cation treatments (below) of experiment II.

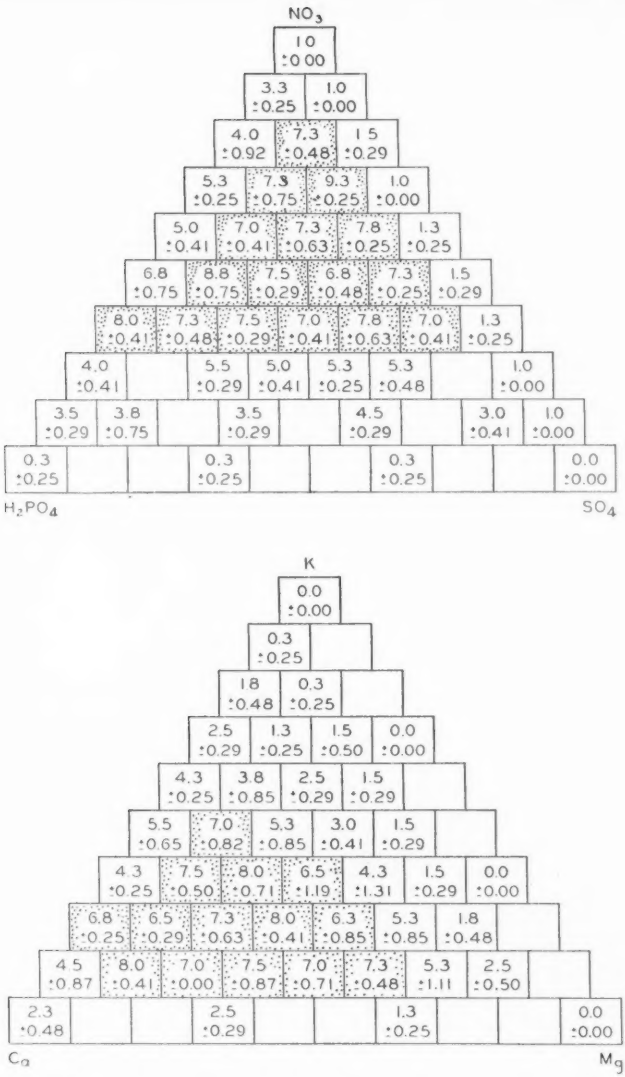

FIG. 11.—Number of fruit which ripened per plant for anion treatments (above) and cation treatments (below) of experiment II.

This experiment had been specifically designed so that data obtained for any character could be analyzed for the presence or absence of statistically significant differences. Accordingly, analyses of variance were computed for each individual character, and the results are compiled in table 2. Since one replication in one

TABLE 2

RESULTS OF ANALYSES OF VARIANCE ON CHARACTERS FOR WHICH DATA WERE RECORDED

CHARACTER	F VALUE	
	BETWEEN TREATMENTS*	BETWEEN REPLICATIONS†
A. Vegetative growth		
1. Height of plants (weekly measurements)	119.71	14.89
2. Number of internodes	71.99	0.08
3. Average length of internodes	14.44	26.58
4. Fresh weight of vine	170.88	2.54
5. Dry weight of vine	133.70	4.87
6. Percentage dry matter of vine	22.77	21.72
7. Dry weight of root system	5.32	2.60
8. Top-root ratio	10.02	7.73
B. Fruit production‡		
1. Total fresh weight of fruit produced	101.51	1.70
2. Total number of fruit produced	60.03	1.21
3. Average fresh weight of fruit (regardless of state of maturity)	44.62	1.51
4. Mean number of fruits which ripened per plant	31.85	5.50
5. Average fresh weight of mature fruit	33.45	0.85
6. Number of days from planting to date of first fruit ripe	11.91	0.79
C. Vitamin C content of first two mature fruits on each plant	5.47	5.27

* An F value of 1.34 is required for a P value of 0.05; when F = 1.49, P = 0.01.
† An F value of 2.64 is required for a P value of 0.05; when F = 3.86, P = 0.01.
‡ All fruits infected with blossom-end rot discarded from this experiment.

treatment was discarded as a result of mechanical injury, YATES' formula (9) was used for the estimation of the missing plot in order to maintain orthogonality. Thus the sources of variations are:

VARIATION DUE TO:	NO. OF DEGREES OF FREEDOM
Treatments	86
Replications	3
Treatments × replications (error)	257
Total	346

This procedure was followed for all characters except height of the plants. In this character the data were further reduced in order to furnish additional information.

It is evident from table 2 that treatments produced statistically significant differences in all the characters listed. It is also evident that differences in environment—when measured by replications—produced significant differences in such characters as height of plant, average length of internodes, dry weight and percentage dry matter of the vine, top-root ratio, mean number of fruits ripening per plant, and ascorbic-acid content of the fruits. This means that replication and randomization were essential to the design of the experiment. Since replication differences were measurable in the analysis of variance, and since these differences were not included in inter-treatment comparisons, valid differences exist which can be ascribed directly to the treatments.

The analysis of variance for height of plants provides one other point which is pertinent to the interpretation of results. In this character, where 12,528 measurements were involved, the validity of a treatment × replication interaction was tested. A significant F value of 1.41 (when $F = 1.23$, $P = 0.01$) was obtained for this source of variation, which means that treatments do not react equally to differences in environment.

The data obtained in the anion treatments show uniform trends in all the characters present. The shaded portions of the triangles represent those treatments where maximum growth or fruitfulness was obtained. It is evident that, in general, the same treatments fall in the shaded portion for every character. These treatments contain 14.2–17.0 milliequivalents of nitrate per liter of nutrient medium. At lower concentrations of nitrate growth and fruitfulness were correspondingly less. In the nitrate-deficient treatments little growth was obtained after 32 days (fig. 5). However, some growth subsequent to this time is shown in the growth curves, which may be indicative of nitrogen reutilization.

When phosphate was absent in the nutrient media, growth and fruitfulness were poor. In this experiment it is evident that 2.8 milliequivalents of phosphate (one-ninth of the phosphate stock solution) was sufficient to result in a maximum expression of any character under conditions of comparatively high nitrate and low sulphate supply. Plants grown in sulphate-deficient treatments were the most productive of any grown in the anion deficiencies. In treatment 22, the plants were as tall as any in the anion triangle, and mean values for other characters were 60–100 per cent of maximum. Treatments where maximum values were obtained for all characters (shaded areas) contained 2.8–8.5 milliequivalents of sulphate.

Since growth and fruitfulness were limited by nitrate supply in most of the treatments, the effects of the higher concentrations of sulphate and phosphate may have been concealed. In sulphate-deficient treatments the higher nitrate and low phosphate concentrations were detrimental. This does not conform to the general effect of nitrate supply and will be emphasized in the discussion of

ascorbic-acid values. Treatments 9, 13, and 14 give maximum values for all characters, while certain other treatments have resulted in comparable values for some of the characters and not for others. Thus, in treatment 22, maximum fruitfulness and growth in height were attained, but fresh and dry weights of vines were significantly less. The sulphate-deficiency symptoms which occurred in these plants give supporting evidence that treatment 22 was not optimum. When many characters are evaluated, it is possible to demonstrate statistically significant differences for small variations in ionic concentrations of the nutrient medium. It is also possible—within relatively narrow limits—to determine the optimum composition of the solutions used.

The interpretation of treatment effect in the cation triangle depends upon the character under consideration. In general, variations of growth in height among treatments followed the same trends as were obtained for fresh weight of vines and dry weight of vines and roots. This group of characters is therefore discussed as a unit and contrasted with fruitfulness where different trends are apparent.

Calcium deficiencies resulted in the smallest plants produced in any of the cation treatments. When as small an amount as 2.8 milliequivalents of calcium per liter was present in the nutrient medium (shaded areas), growth in height and fresh and dry weights were maximum. This is the lowest concentration used in any treatment containing calcium. The concentration of potassium and magnesium in optimum treatments varied from 5.7 to 17.0 milliequivalents per liter, and variations in their relative proportions produced no significant effects within these ranges. The effect of treatment on the dry weight of the roots is discussed in connection with a consideration of the percentage dry matter. In potassium-deficient treatments—unless calcium was absent—growth was approximately 40 per cent of maximum when height of plant was used as a criterion. The fresh weight of vines and dry weights of vines and roots were relatively less, with no values exceeding 10 per cent of maximum. With magnesium deficiencies, certain treatments produced much more growth than others, depending upon the relative proportions of calcium and potassium present. Plants of treatment 69 were 80 per cent as tall and produced 30–40 per cent as much fresh and dry weight as the optimum treatments. It may be noted that treatments deficient in both potassium and magnesium resulted in relatively tall plants with comparatively little fresh or dry weight.

The data for fruitfulness may be contrasted with the observations for other characters. Nutrient treatments resulting in maximum values for total weight of fruit per vine contained the following milliequivalents per liter of major cations: 11.3–17.0 of calcium, 2.8–8.5 of magnesium, and 5.7–11.3 of potassium. The greatest number of fruits ripening per plant occurred over a wider range of concentrations but include the preceding optimum treatments. Other data, including

number and average weight of fruit produced, support the generalization that fruit production is greatest in high calcium treatments. As the calcium content of the nutrient medium was less, fruitfulness was correspondingly less; but at low concentrations of calcium, fruit production was greater in those treatments containing relatively low potassium and high magnesium. It is apparent that plants grown in treatment 69 (deficient in magnesium) were approximately 70 per cent as fruitful as any in the cation triangle, and it will be recalled that these plants also produced relatively large vines. Optimum fruitfulness and optimum vine growth have not occurred in the same nutrients. However, total dry weight production (vine+roots+fruit) was greatest in treatments where best fruitfulness was observed.

It is evident from table 2 that detailed data for many characters are not presented. However, the data for percentage dry matter in vines furnish several pertinent correlations and are given in figure 12. In general, there is an inverse correlation between growth and fruitfulness and percentage dry matter. Significantly high values for the latter character were obtained in all deficiency treatments (except in some deficient in sulphate and in one deficient in magnesium). In treatment 48, the percentage dry matter of the vines was significantly less than in any other treatment. Figure 9 shows that maximum dry-weight accumulation in root systems also occurred here. There is no obvious explanation for this correlation.

Certain correlation coefficients contribute to the interpretation of the data. From table 2 it is apparent that statistically significant differences between treatments were demonstrable for the average length of internodes as well as for the number of internodes. The average length of internodes was significantly less when plants were grown in nitrate-deficient solutions and was significantly greater in treatments deficient in potassium (except the treatment also deficient in calcium) and in those treatments where maximum growth in height occurred (50, 51, 55, 56, 61, 67, 75). The greatest number of internodes coincided with the greatest height of plants in the anion triangle; but such was not the case in the cation triangle, where maximum values were obtained in treatments 50, 54, and 55. Further reduction of the data therefore seemed desirable. Assuming x = height of the plant, y = number of elongated internodes, and z = average length of elongated internodes, then

$$\text{(a)} \quad r^{5}\,xy = +0.977$$
$$\text{(b)} \quad r\,xz = +0.268\,.$$

With the respective number of degrees of freedom involved (85), a value of 0.275 is required when P = 0.01, and thus a significant correlation exists in equation (a) and is not demonstrable in equation (b). According to P values arbitrarily re-

[5] r, Correlation coefficient between indicated variables.

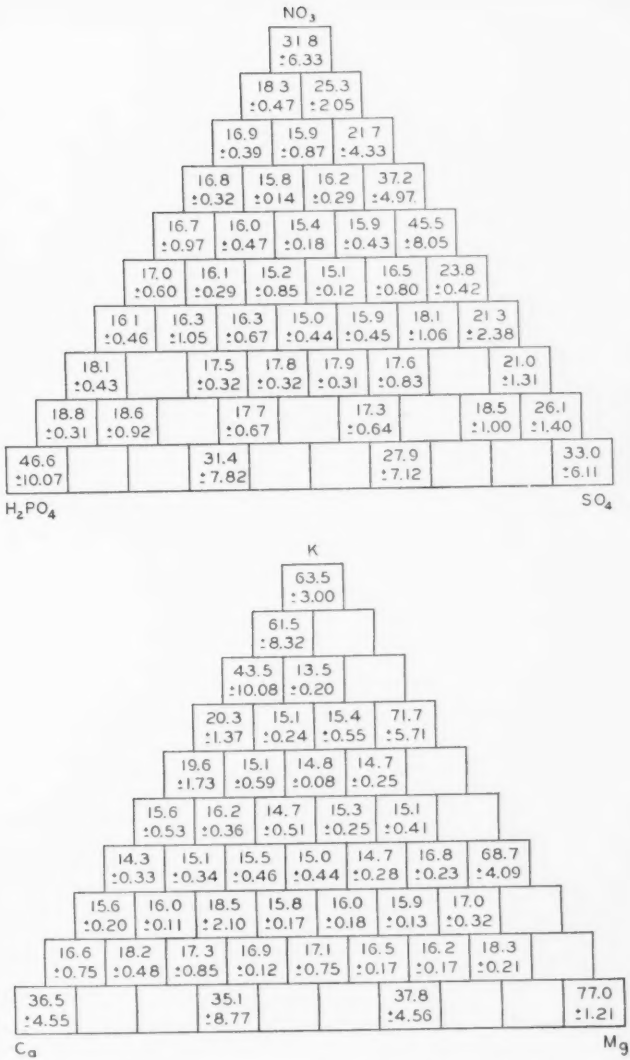

FIG. 12.—Percentage dry matter of vines for anion treatments (above) and cation treatments (below) of experiment II.

quired in this experiment, no general correlation occurred between height of plant and average length of elongated internode, while a highly significant correlation occurred between height of plant and number of internodes. This is reasonable considering the variation in response obtained, and it is surprising that significantly longer internodes occurred in certain treatments.

Data on top-root ratio, total number and average weight of fruit, and earliness support in general the other observations that growth and fruitfulness are significantly affected by the variations in nutrient composition in this experiment. It is felt that individual discussion of these characters is not necessary.

Ascorbic-acid determinations were made on 1447 fruits in this experiment, and the results are presented in figure 13. From table 2 it is evident that statistically significant differences between treatments occur for this character. However, no significant differences were obtained between any of the treatments not deficient in one or more of the macronutrient elements. Deficiencies in potassium and phosphate resulted in significantly less ascorbic-acid content, while treatments deficient in nitrate resulted in relatively high values but few fruit were produced, and the results are not statistically different from the other treatments. No fruits were analyzed in treatments deficient in calcium, since none matured. Significantly higher ascorbic-acid values were obtained in treatments 2, 4, 7, and 11, which were deficient in sulphate. These treatments were relatively high in nitrate and low in phosphate, and it was pointed out in the discussion of fruitfulness that such a condition was detrimental.

Although significant mean differences in ascorbic-acid values are demonstrable, the magnitude of the differences is not great. These conditions of extreme nutrient deficiency are probably rarely encountered in commercial production. The important fact is indicated that a macronutrient supply adequate to produce a fair yield is not a limiting factor in ascorbic-acid content. In situations where extreme deficiencies of potassium and phosphate seriously limit production, fertilization may increase ascorbic-acid content as well as yield.

While many data obtained in experiment I have not been included in this paper, it should be emphasized that the same trends were apparent in both experiments. The maximum values for growth and fruitfulness occurred with identical conditions of nutrient composition, and—as in experiment II—the highest ascorbic-acid value in experiment I resulted from sulphate deficiency. In the latter experiment, however, the plants produced more fresh and dry weight and were far more fruitful. Average ascorbic-acid values were also twice as high.

Experiment III

In spite of the fact that the ascorbic-acid content of the fruits from various treatments was remarkably uniform in each of the previous experiments, a wide

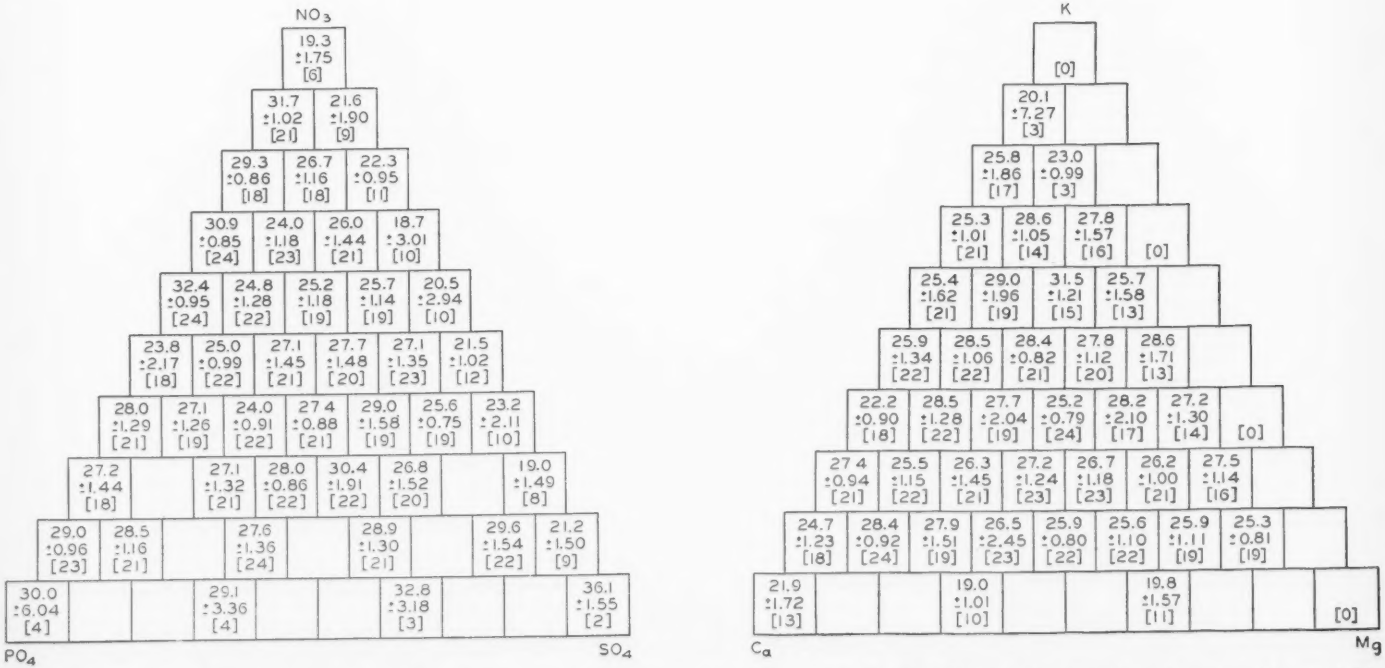

FIG. 13.—Ascorbic-acid values in experiment II, giving treatment means together with their standard errors. Number of analyses in each treatment is recorded in brackets. Left, anion triangle and right, cation triangle.

variation was noted between the average values of the two experiments. Since the first experiment was conducted in the greenhouse during the fall and winter months and the second was out-of-doors during the summer, it seemed possible that climatic environmental factors might have been primarily responsible. The purpose of experiment III was to measure the influence of soils and of variations in environment associated with the top of the plant on the ascorbic-acid content of the tomato fruit.

METHODS.—Five widely separated locations in the United States having different climatic conditions were selected. These locations included Ithaca, New York, Riverside, California, Berkeley, California, Cheyenne, Wyoming, and Lake Geneva, Wisconsin.[6] The inbred strain of Bonny Best tomatoes used in experiment II was grown in good garden soil at each location. The soil was undisturbed at Riverside and Lake Geneva but was confined to large containers at all other locations. The plants were grown out-of-doors, except at Berkeley, where the plants were grown in a well-lighted greenhouse. A large sample of soil from three of the locations was shipped to Ithaca, and the same strain of tomatoes was grown in each of the soils. As nearly as possible the same fertilizer and cultural practices were followed for a particular soil sample at Ithaca as was used for the soil at its original location. At the same time at each location the same strain of tomatoes was grown in sand culture, and Hoagland's nutrient solution (**4**) was used. At all locations the vines were pruned of all axillary growth and supported by stakes. The fruits were picked at each location when completely red and analyzed for ascorbic acid as previously described. Thus two sources of variation on the ascorbic-acid content of tomato fruits were tested: (*a*) differences between climates when plants are grown in naturally occurring soils and in sand cultures; and (*b*) effects of different soils in the same environment. The design also permitted a comparison of soil and sand culture as it affected ascorbic-acid content.

RESULTS AND DISCUSSION.—The ascorbic-acid analyses of fruits from plants grown in naturally occurring soils and in sand cultures at the various locations are given in table 3. It is evident that fruit produced on plants grown in naturally occurring soils or in sand culture at various locations varied in ascorbic-acid content. Mean differences well in excess of three times their standard errors are demonstrable, and hence the variation is significant. At Ithaca, Riverside, and Berkeley only slight differences in the ascorbic-acid content of the fruit were observed when soils and sand cultures were compared. In all three locations the mean differences were not of sufficient magnitude to demonstrate P values of 0.01 in a *t* test and hence are not considered as significant.

[6] The writers gratefully acknowledge the collaboration of Drs. H. E. Hayward, D. R. Hoagland, and A. C. Hildreth.

At Ithaca no special sand culture was set up using Hoagland's solution, since in experiment II a large number of sand cultures were available for comparison with the soil. The figures used in table 3 were those analyses obtained for fruit ripening during the same time interval that fruits were harvested from the soil-culture plots. However, since Hoagland's solution calls for the use of ammonium salts, a separate comparison was later made between solution 25 of experiment II and Hoagland's solution. No significant difference in ascorbic acid was noted between the two treatments, so it is assumed that this comparison is valid.

TABLE 3

ANALYSIS OF ASCORBIC-ACID CONTENT OF TOMATO FRUITS FROM PLANTS GROWN IN SOIL OR IN SAND CULTURE UNDER DIFFERENT ENVIRONMENTS. VALUES EXPRESSED AS MILLIGRAMS OF ASCORBIC ACID PER 100 GM. FRESH FRUIT

Location	Plants grown			
	In naturally occurring soils		In sand culture	
	Mean ± SE	No. of analyses	Mean ± SE	No. of analyses
Ithaca, New York	24.7 ± 0.94	17	24.6 ± 1.13*	30
Riverside, California	21.2 ± 0.65†	23	22.5 ± 0.92	17
Berkeley, California‡	18.0 ± 0.41	14	16.0 ± 0.75	18
Lake Geneva, Wisconsin	23.7 ± 0.67†	15	30.5 ± 1.12	15
Cheyenne, Wyoming	27.1 ± 1.34	20	.....	.....

* Plants supplied with nutrient solutions of experiment II. Analyses are of fruit which ripened during same time interval as those from soil plot.

† Plants grown in undisturbed soil.

‡ Plants grown in the greenhouse.

At Lake Geneva, however, a significant mean difference of 6.8 ± 1.31 mg. was observed for the ascorbic-acid content of fruits on plants grown in soils and those grown in sand culture. The explanation for this discrepancy is not known, but the following observations may be pertinent. During the early growth of the plants at this location, the sand cultures were watered liberally with nutrient solution, and the plants grew very rapidly (more so than comparable plants in soil). The blossom set of the first blossom clusters was very poor, most of the clusters falling from the plant soon after bloom. The general appearance of the plants indicated an over-abundant supply of nitrogen. In order to assure a good set of fruit, therefore, nutrient solution was withheld from the plants for varying periods of time after first bloom, and the plants were watered with spring water. Nutrient solu-

tion was supplied from time to time to assure adequate growth and good fruit set. These plants grown in nutrient solution at Lake Geneva produced more luxuriant growth than did the plants in sand culture at any other location.

The preceding data indicate that fruit produced on plants growing in a good soil may have the same ascorbic-acid content as fruit produced on plants growing in a complete nutrient solution. The variations which occurred between the two treatments at the one location (Lake Geneva) indicate that the technique of applying nutrient solutions may possibly be important.

The results of ascorbic-acid analyses of fruits from plants grown in different soils, but under the same conditions of climatic environment, are given in table 4. It is evident from the data that the soils used in this experiment do not sig-

TABLE 4

ASCORBIC-ACID CONTENT* OF FRUITS ON PLANTS GROWN IN DIFFERENT SOILS BUT IN SAME CLIMATIC ENVIRONMENT AT ITHACA

SOIL FROM	MEAN ± S.E.	NO. OF ANALYSES
Ithaca, New York	24.7* ± 0.94	17
Berkeley, California	24.1 ± 1.12	16
Cheyenne, Wyoming	24.4 ± 0.94	21
Lake Geneva, Wisconsin	26.0 ± 1.76	11

* Mg. ascorbic acid per 100 gm. fresh weight of fruit.

nificantly alter the ascorbic-acid content of tomato fruits under conditions of the same climatic environment. It is realized that the properties of soils are altered when they are disturbed in transporting from one location to another, and there seems to be no way to correct for this factor. It should be recalled, however, that the soil at three of the locations was placed in large containers, and the handling involved probably represented as much disturbance as was involved in sending comparable soils to Ithaca.

A comparison of the ascorbic-acid values shows much greater differences between fruits produced at different locations than between fruits produced on plants in the different soils at a single location. In fact, all the soils used at Ithaca produced fruit of equal ascorbic-acid content. On the other hand, at Berkeley the average value was 18.0 ± 0.41 mg. per 100 gm., while at Cheyenne the values were 27.1 ± 1.34 mg. 100 gm. Thus the location where the plants are grown may produce a significant influence upon the ascorbic-acid content. While the plants at Berkeley produced fruit relatively low in ascorbic acid, it should be noted that these plants were grown in the greenhouse. Results from experiment IV have a bearing on this point.

In this connection, it should be noted that environment within a given climate significantly affects the ascorbic-acid content of the fruit. From experiment II (table 2) it is evident that significant differences between replications occurred for this character.

When the ascorbic-acid content of fruits produced in selected rows from east to west in the experimental plot was computed, values of 24.6 ± 0.85, 26.8 ± 1.06, 27.9 ± 0.93, and 29.3 ± 1.02 mg. per 100 gm. fresh weight were obtained. Each of these values represents forty or more analyses, and the plants on the east side of the plot produced fruit significantly low in ascorbic-acid content.

Experiment IV

During the course of the three previous experiments, several additional investigations, relatively simple in design, were carried out. Most of them were conducted concurrently with experiments II and III, and some of the data of the latter are used for comparisons.

At the time the plants were grown out-of-doors at Ithaca in the four different soils, comparable plants were grown in the greenhouse in the same soils. The average ascorbic-acid content of the sixty-five fruits analyzed from the plots outside was 24.7 ± 0.18 mg./100 gm., while the average of fourteen analyses of fruits in the greenhouse was 23.9 ± 1.46. It is evident from these values that the ascorbic-acid content of fruits produced in the greenhouse is not significantly different from that of fruit produced out-of-doors at the same time of year. The great differences in the acid content of fruits between experiments I and II cannot be ascribed merely to the fact that one was carried out in the greenhouse. Two different sources of seed were used in the two experiments, and it was thought that genetic differences between the plants might explain the variation in ascorbic-acid values. To test this possibility, plants of the same inbred strain as used in experiment II were grown in the greenhouse during the fall and winter of 1941. They were grown in sand culture and supplied with a complete nutrient (treatment 25, fig. 4). Fruits were harvested during late November and early December. The average ascorbic-acid content of twelve fruits was 12.2 ± 0.20 mg. per 100 gm. fresh fruit. These values closely approximate those obtained in experiment I, indicating that the ascorbic-acid content is dependent to a large extent upon the season in which the plants are grown.

Several miscellaneous observations were made during the course of the experiment. At Lake Geneva, besides the two sets of plants grown in sand culture and in soils, a third set of comparable plants were grown in soils but the vines were not staked or pruned. These plants grew luxuriantly and produced the heaviest crop of fruit of any of the three lots. The average ascorbic-acid content of these fruits was 20.5 ± 0.78 mg. per 100 gm. (fifteen analyses). When this value is compared

with a similar value for fruits from supported vines (23.7 ± 0.67 with fifteen analyses), a significant mean difference of 3.2 ± 1.03 ($t = 3.11$) is demonstrable. While supporting the vines at this location significantly increased the ascorbic-acid content of the fruit, the magnitude of the differences was not great.

General discussion

These results have indicated that minor variations in the relative concentrations of the macronutrient elements in the nutrient medium may exert a marked influence on the growth and fruitfulness of tomatoes. In general, variations in calcium and nitrate produced more apparent differences over wider ranges of concentrations than were obvious with the other elements. The tallest plants were correlated with low concentrations of calcium, but greatest fruitfulness occurred at calcium levels which were four to six times as high. Similarly, as the concentration of nitrates was increased from 0.0 to 8.5 milliequivalents per liter, growth and fruitfulness were correspondingly greater. While marked variations in plant response could be directly correlated with differences of from 0.0 to 2.8 milliequivalents of the other macronutrient elements, it is evident that some effects were produced by other ranges in concentration. It is difficult, however, to relate these effects directly to variations in the concentration of any individual ion. Thus, at calcium concentrations of 5.7 milliequivalents, much greater fruitfulness resulted when potassium was relatively low and magnesium relatively high. At this same calcium level, however, greatest growth in height occurred when potassium was relatively high and magnesium relatively low. Other examples could be cited which would also illustrate the interaction of ionic effects, even in treatments completely lacking one or more ions.

The results of ascorbic-acid determinations have shown little correlation with variation in macronutrient supply, even though growth and fruitfulness were markedly affected. This may indicate that little variations in ascorbic-acid content would be expected to result from soil differences in so far as such differences are the result of macronutrient supplying power. This is supported by the fact that the four soils tested had no effect. Other soil differences, however, such as physical structure or the ability to supply micronutrients, might be important.

Ascorbic-acid content was found to be correlated with climatic environmental differences associated with season and location. Such differences might include variations in length of day, light quality, light intensity, temperature, etc., and these possibilities are being investigated at the present time.

It seems likely that the effects of climatic environmental factors upon ascorbic acid may have considerable practical importance, especially since many of the fruits consumed during the course of the year are produced in greenhouses during the winter. If, as these results indicate, winter-grown fruits are consistently 50 per

cent lower in ascorbic acid than summer fruit, then the value of tomatoes as an anti-ascorbutic agent would be relatively low during the season when the problem of getting an adequate supply of the vitamin in the diet is more difficult. If other varieties behave in a manner similar to this particular strain of Bonny Best and in the winter produce only 12 mg. per 100 gm. of fresh fruit, then the average ascorbic-acid values for tomatoes in various food tables would have to include separate values for summer- and winter-grown fruit. In these experiments winter-grown fruits are lower in ascorbic acid than many canned products which are prepared from summer-grown tomatoes.

Summary

1. Four experiments were carried out to measure the influence of environmental conditions on the ascorbic-acid content of Bonny Best tomatoes. Two of them were designed to test the influence of macronutrient supply and the others measured the influence of climatic environmental factors.

2. In sand cultures, growth and fruitfulness could be correlated with minor variations in nutrient composition.

3. Gross appearances of plants grown in eighty-seven different solutions are discussed and quantitative data presented for growth and fruitfulness. These data are reduced and analyzed by statistical methods.

4. In general, variations in calcium and nitrate in the nutrient medium produced greater differences in growth and fruitfulness of plants over wider ranges of concentrations than were obvious with the other elements. Interactions of the effects of various elements were noted.

5. Ascorbic-acid content of the fruit was significantly higher in some sulphate-deficient treatments and significantly lower in potassium and phosphate deficiencies than average values. With these exceptions, no demonstrable effect of mineral nutrient supply on ascorbic acid was observed.

6. Plants grown during the fall and winter produced fruit with approximately one-half as much ascorbic acid as was produced by comparable plants during the summer.

7. The location where the plants were grown had an effect upon the ascorbic-acid content of the fruit. These effects could not be correlated with differences between soils at the several locations and were apparently associated with differences in the environment of the top of the plant. Significant positional effects on ascorbic-acid content, even in a relatively small trial plot, were observed.

8. Plants grown in sand culture supplied with a balanced nutrient solution produced fruit as high as or higher in ascorbic-acid content than that obtained through the use of a good soil.

9. Several other observations are included relative to the effects of variations

in mineral nutrition on growth and fruitfulness and the influence of environment upon ascorbic-acid content.

10. A discussion of the possible practical significance of the results obtained is included.

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CHROMOSOME FRAGMENTS IN LILIUM WILLMOTTIAE AND HYBRIDS BETWEEN IT AND L. DAVIDII[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 540

J. M. BEAL

(WITH NINE FIGURES)

Chromosome fragments have been reported in *Lilium japonicum* (2, 3, 4, 5), *L. henryi* (2, 3, 4), and *L. formosanum* and *L. tigrinum* (4). *L. willmottiae* can now be added to the list, since more than 95 per cent of the microsporocytes in smear preparations from plants of this species growing at Wychwood, Lake Geneva, Wisconsin, have shown one fragment in addition to the twelve normal bivalents.

The fragment is easily detectable at diakinesis and at late metaphases of meiosis I (fig. 1), since it generally passes intact to one pole of the spindle before the bivalents disjoin. At late anaphase of meiosis I (fig. 2) it lies near the attachment constriction region of the anaphasic group of chromosomes and later is included with the chromosomes of this dyad nucleus when the nuclear membrane forms.

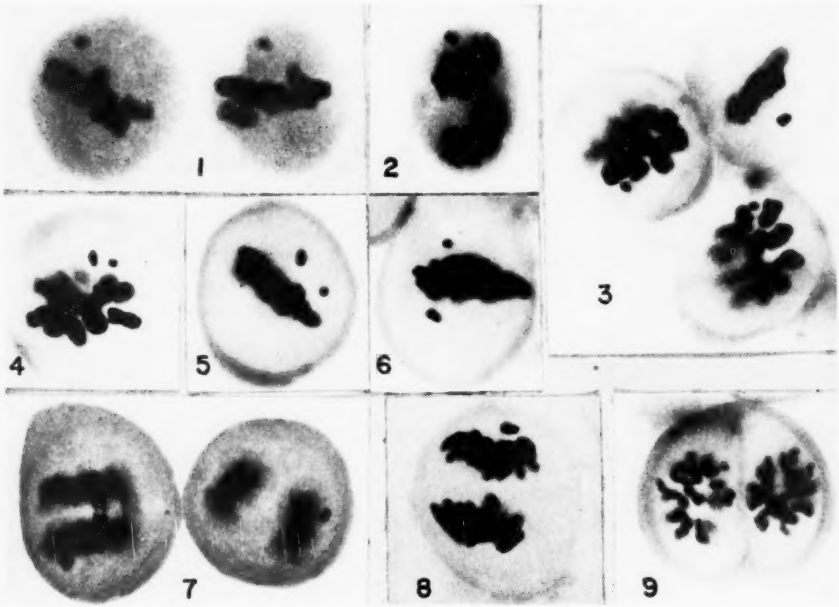
The behavior of the fragment during meiosis II has not been determined. The fragment is short and compact and its structure obscure. In several instances it has appeared as a compact double body as it passes toward the pole in meiosis I; hence it is possible that it may divide in meiosis II and thus provide a fragment for two of the four quartet nuclei and microspores. It may not divide in meiosis II, however, and in this event only one of the four microspores would possess the fragment in addition to the haploid number of twelve chromosomes.

The point of immediate interest associated with the fragment is that it is found in certain hybrids between *L. willmottiae*×*L. davidii* and the reciprocal cross. Microsporocytes from a single slide of *L. davidii* have failed to show fragments in this species during diakinesis and metaphase I. This is not decisive proof of their absence, but evidence from the hybrids to be mentioned shortly indicates the nonoccurrence of fragments in this species.

During the summer of 1937, crosses were made between *L. willmottiae*×*L. davidii* and *L. davidii*×*L. willmottiae*. Viable seeds were obtained as a result of both crosses and numerous seedlings have been established. All the seedlings resemble *L. willmottiae* much more closely than the other parent, and the hybrids are larger than either parent. Many of them produced flowers in the summer of

[1] This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

1940 and again in 1941. Anthers from a number of the flower buds were smeared, and the microsporocytes fixed in Navashin's solution and stained according to the gentian violet-iodine method, but no record was kept of the individual plants from which the various slides were made. Twelve of these slides show either first or second meiotic divisions, six being from the *L. willmottiae*×*L. davidii* cross and six from the reciprocal. Of the twelve slides, one shows no fragments in any of the



FIGS. 1–9.—Fig. 1, *L. willmottiae*, metaphases I. Fig. 2, same, late anaphase I. Fig. 3, *L. willmottiae*× *L. davidii*, metaphase I. Figs. 4–6, same, metaphase I, two fragments in each sporocyte. Figs. 7, 8, *L. davidii*×*L. willmottiae*, anaphases I. Fig. 9, same, metaphase II. Fragment in left dyad at right of upper center.

sporocytes. This slide was made from one of the plants resulting from the *L. davidii*×*L. willmottiae* cross, which indicates that fertilization had been accomplished by a gamete from a pollen grain which did not possess a fragment. One slide from the *L. willmottiae*×*L. davidii* cross showed two fragments (figs. 4–6) of unequal sizes, while the remaining ten slides representing both crosses all show one fragment in practically every sporocyte (figs. 3, 7–9).

The origin of the second fragment is unknown and it is smaller than the one which regularly occurs in *L. willmottiae*. Both fragments (fig. 5) may pass to the same pole at metaphase I, or they may go one to each pole (fig. 6). Their behavior

in meiosis II has not been determined. In all the hybrids which contain a single fragment in the sporocytes the fragment behaves as described for *L. willmottiae*.

The occurrence in *L. willmottiae* of the fragment which is passed, apparently unchanged, to hybrid progeny—whether *L. willmottiae* is used as the seed or the pollen parent—may perhaps be unexpected and raises several questions which cannot at present be answered. For example, does the fragment possess an attachment constriction? Its regular passage to one pole of the spindle and its subsequent inclusion in one of the dyad nuclei suggest that it may, but its compactness at diakinesis and metaphase I makes it extremely difficult to determine. In numerous instances the fragment has appeared to be double at anaphase I, thus suggesting that it may divide at meiosis II. It is possible, however, that no spindle attachment is present and that the fragments may behave in the same manner as X-ray-induced fragments in the neuroblasts of *Chortophaga viridifasciata*, reported by CARLSON (1). These fragments, lacking spindle attachments, show behavior and distribution similar to the chromosomes, indicating that polar repulsions effect the equatorial position at metaphase; that the separation of the chromatids is autonomous; that the orientation and migration toward the poles is due either to protoplasmic streaming or to an expanding "Stemmkörper"; and that the attachment constriction functions mainly in making the orientation and separation of the chromosomes uniform and equal. Whether the observations of CARLSON are applicable to the *Lilium* species and hybrids reported here will have to await the outcome of more detailed studies now under way.

The fragments have shown no evidence of being reduplications of sections of the normal chromosomes, such as MATHER (3) reported for *L. japonicum* and *L. henryi*, since in none of the sporocytes examined has there been any indication of pairing between the fragment and a major chromosome.

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TOXIC EFFECTS OF SODIUM PENTACHLORPHENATE AND OTHER CHEMICALS ON WATER HYACINTH

A. A. HIRSCH

In many streams of southern United States profuse growths of water hyacinth (*Crassipes eichornia*) impede navigation, retard drainage flows, favor mosquito breeding, and prevent fishing. Eradication has been confined to treatment with hazardous arsenicals or to mechanical processes, such as removal by raking or the recent maceration development.

The experiments described here were the preliminaries in a search for a safe poison to control this growth in earth-lined drainage canals near an urban area. Of particular interest for trial were two recently introduced materials: sodium pentachlorphenate, an industrial algaecide and fungicide marketed as Santobrite by the Monsanto Chemical Company of St. Louis, Missouri, and Beneclor 3, a chlorinated hydrocarbon, manufactured by the Chloroben Corporation, Jersey City, New Jersey. Copper sulphate was also included for observation. A control set completed the system.

Seventy-eight mature plants were collected from a nearby bayou and divided equally into four 14-gallon, painted, iron washtubs stored outdoors and almost filled with water. River silt was placed in the bottoms of the tubs. Santobrite and copper sulphate were added every 2 weeks from a stock solution to give increments varying from 5 to 20 p.p.m.; beneclor 3, a liquid, was pipetted directly in amounts equivalent to 5–10 gallons per acre. The toxins were thoroughly mixed with the water in the tubs. The amount of additional dosage was decided from the physical appearance of the plants. This incremental treatment was adopted in order to establish the approximate lethal limits employing the minimum number of experimental units. The experiment terminated in advance of cold weather to avoid killing by the first freeze. The results are summarized in table 1.

Transfer of plants from their natural habitat to the tubs caused some stunting; nevertheless all plants remained healthy, unless poisoned, and produced flowers at various times. Dwarfing was especially noted at low initial doses of santobrite, but specimens treated with low dosages of copper sulphate and beneclor 3 seemed to thrive even better than the controls. When concentrations of 45 p.p.m. copper sulphate and 45 gallons per acre of beneclor 3 were reached, these sets were abandoned because the toxicity was insufficient to warrant further observation. While some damage was inflicted by beneclor 3, the lethal dose on 2 weeks' con-

tact was probably much higher. Even the initial dose of santobrite caused yellowing of stalks and withering of leaves; further applications damaged tissues more definitely and dwarfed new leaves. About 35 p.p.m. seemed to arrest growth, but complete necrosis was obtainable only after reaching a concentration of 80 p.p.m. This strength is about 2.7 times the maximum recommended for control of algae

TABLE 1

SUMMARY OF RESULTS

Dosage	Control	Copper sulphate	Beneclor 3	Santobrite
Smallest necessary to affect appearance		35 p.p.m.	35 gal./acre	5 p.p.m.
Necessary for complete kill		Not reached	Not reached	80 p.p.m.
Maximum reached		45 p.p.m. (abandoned)	45 gal./acre (abandoned)	80 p.p.m.
Final appearance of survivors	Good condition; few brown spots	Like control except more widespread browning	Two nearly dead; others extensively necrotic	All dead
	(all original plants living)			

in industrial cooling systems and is too low to cause skin irritation, even on prolonged contact.

The physiological action of santobrite suggests two field procedures for combating the problem of the water hyacinth: (1) A light application, somewhat more than 5 p.p.m., for retarding growth in areas where complete removal is not essential or in zones where other aquatic life is to be preserved. (2) Complete elimination by a dosage approximating 80 p.p.m. This method is applicable especially to reach into shallow or inaccessible tributaries in connection with a maceration unit.

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EFFECT OF HEAT-DRYING UPON THE PERIDERM OF WASHED POTATOES[1]

RICHARD O. BELKENGREN AND EDWIN S. CIESLAK

For some years it has been the practice of commercial potato growers to wash the tubers prior to shipment. This removes the adhering soil as well as permitting a more thorough rejection of those obviously defective or rotten. The usual procedure has been to convey the potatoes through a pre-sizer, then to a washing tank or spray chamber, and finally to a picking table discharging into sacks or crates. Loading wet or damp potatoes has resulted in considerable rot in transit, and drying equipment has been installed at some packing houses. As a rule, the drying immediately follows the washing and precedes the picking.

In the operation of one particular drying installation (1), it was noticed that the potatoes when shipped were extremely resistant to spoilage, both in transit and subsequent storage. When more than 1000 carloads of potatoes subjected to this treatment resulted in no spoilage claims whatever, it was concluded that the protection afforded could not be accounted for solely on the basis of external moisture removal. The drying procedure subjects the potato to a moderately high velocity movement of hot air in the course of passage through the unit. That the effect was not due entirely to removal of inoculating organisms was established by experiments in which the tubers were artificially inoculated externally with various organisms which might cause spoilage. With other conditions remaining constant, the treated potatoes continued to show greater resistance to rot than did the controls, indicating the probability of a biological change within the tuber itself.

The potato periderm consists of three parts: the phellem, several layers of heavily suberized cells very much flattened; the phellogen; and the phelloderm, several layers of cells lying next to the cortical parenchyma. The cell walls of these layers protect the inner portions. In freshly harvested potatoes, only a few layers of cells are present, but with time the number of cell layers increases. This increase is affected by a number of factors, chief of which are temperature and moisture.

Two varieties, a white potato and a red one, Bliss Triumph, were used in the experiments. Samples were obtained from commercial driers and from laboratory-dried tubers prepared under similar conditions. Controls consisted of samples taken immediately before and immediately after treatment. Other samples were taken 3–6 days after treatment, in order to show the change.

[1] This work was done under the sponsorship of F. C. Peters, grower, Goulds, Florida.

Pieces of tissue 6 mm. square by 3 mm. deep were cut from the surface of the tubers. These were killed and fixed in Allen-Bouin fluid for 3–6 hours, washed in 50 per cent alcohol, dehydrated in 70, 95, and 100 per cent alcohol, cleared in xylene, slowly infiltrated with paraffin, and finally imbedded in a paraffin block. From each piece of tissue, thirty random 18-μ sections were taken from the middle one hundred, fixed on slides, freed of paraffin, hydrated to 50 per cent alcohol, stained in safranin, washed and destained if necessary, counterstained with Delafield's haematoxylin, dehydrated in successive changes of alcohol, cleared in xylene, and mounted in balsam. Four random sections out of each thirty were measured.

The averages of 1440 measurements and cell counts are given in table 1. Fifteen potatoes were used in each group. These samples were given the indicated heat treatment in a laboratory drying oven.

TABLE 1

RESULTS OF HEAT-DRYING ON POTATO PERIDERM, MEASURED 72 HOURS AFTER TREATMENT

Group	Treatment	Phellem		Phellogen-Pelloderm		Total periderm (μ)	Total no. of cells
		Thickness (μ)	No. of cells	Thickness (μ)	No. of cells		
A	Control	77.82	5.63	63.23	3.93	141.05	9.56
B	63° C. for 8 minutes	82.99	6.18	73.72	4.21	156.71	10.39
C	78.5° C. for 4 minutes	94.24	6.23	106.70	4.77	200.94	11.00

Group B averaged 0.83 cell layers and group C 1.44 cell layers greater than the control (group A). The 4-minute exposure to the higher temperature favored a greater increase in thickness in 72 hours than the longer exposure at the lower temperature. The cell walls in groups B and C were also thicker than those of the controls. This is attributed to increased suberization, probably promoted indirectly by the higher temperature, since storage of potatoes at low temperatures retards suberin formation. Control lots of potatoes dried without heating showed no increase in cells after 72 hours. Immediately following the drying period, the periderm showed a slight decrease in thickness, presumably due to drying.

Recently harvested Bliss Triumph potatoes, treated on a commercial scale by the same heat-drying process, showed an increase in periderm cell layers from 8.7 to 10.9. Results on samples obtained from another similar commercial unit and shipped to this laboratory showed 11.53 cell layers in the periderm of heat-dried potatoes against 10.47 for the controls, an increase of 1.06 layers. Thickening of the phellem cell walls was also noted.

The increase in periderm thickness is especially noted in about 72 hours and appears to be initiated by the drying treatment. The increase and the suberization are marked in young potato tubers having a relatively thin periderm at the start. Obviously the heat which the tuber absorbs in the drier subsequent to removal of its external moisture acts as a stimulant for the formation of periderm. Possibly the change is hormonal in character. Apparently the effect is due, at least in part, to this heat rather than to mere drying alone. In support of this, THORNTON (2) reports that periderm formation normally does not take place so rapidly under dry conditions as in a moist environment. Additional work in progress in this laboratory is now aimed at a fuller understanding of the effect.

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CHROMOSOME NUMBER OF ZANTHOXYLUM AMERICANUM[1]

RUTH I. WALKER

(WITH EIGHT FIGURES)

The haploid chromosome number of 35 was reported for staminate plants of *Zanthoxylum piperitum* by SINOTÔ (2). He observed that the chromosomes of pollen mother cells at metaphase of the first meiotic division are similar in size, except for a few which are larger. One of these larger chromosomes appears to be a univalent and is always found at the periphery of the equatorial plate. He frequently saw, however, an unequal pair of chromosomes. NAKAJIMA (1), working with staminate plants of the same species, also observed that the reduced number of chromosomes is 35. He found an unequal pair of chromosomes and a small univalent.

Because of these reported differences, and since no account has appeared concerning the chromosomes of the American species *Zanthoxylum americanum* Mill., the study reported in the present paper was made.

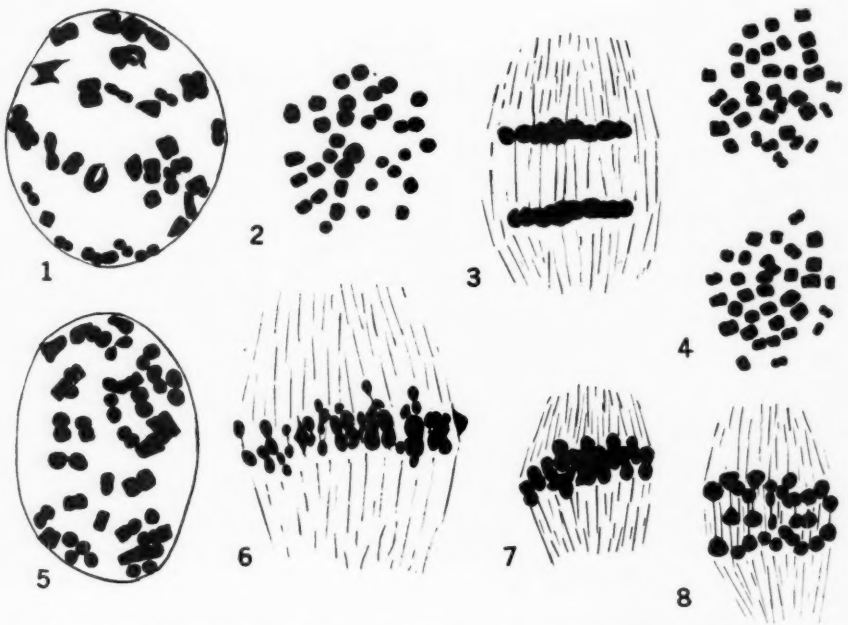
Staminate and pistillate buds were collected from plants of *Z. americanum* near Sauk City, Wisconsin, and in Milwaukee, Wisconsin. The material was fixed in Flemming's medium and Carnoy's acetic-alcohol-chloroform solutions. The fixed material was imbedded in paraffin, cut at 8–12 μ, and stained with Heidenhain's iron-alum haematoxylin or crystal violet.

A study of the pollen mother cells of plants from each locality at diakinesis and at the equatorial plate of the first meiotic division showed 34 pairs of chromosomes (figs. 1, 2). At metaphase these are ovoid and are similar in appearance, except for size. During the anaphases the movement of the chromosomes toward the poles is normal (fig. 3), although occasionally the chromosomes of a pair may lag or reach the poles earlier. At metaphase of the second meiotic division 34 chromosomes are present on each spindle (fig. 4). Fewer preparations were obtained of the macrospore mother cell during meiosis, but in such figures as were found 34 pairs of chromosomes are present (fig. 5).

Studies were also made of lateral views of microspore and macrospore mother cells during first metaphases and anaphases to determine the form and number of the chromosomes. Many preparations were examined, but owing to the size,

[1] This research was done with the assistance of W. H. Falvey of Work Projects Administration, University Natural Science Project, Work Project no. 10324.

number, and crowding of the chromosomes, it was impossible to distinguish all pairs of chromosomes distinctly in one view. Of the chromosomes observed, however, all are paired and the chromosomes of each pair are of the same size and form (figs. 6–8). At no time is there any indication of a univalent or of an unequal



FIGS. 1–8.—*Zanthoxylum americanum:* Fig. 1, pollen mother cells at diakinesis. Fig. 2, polar view of equatorial plate; first meiotic division. Fig. 3, anaphase. Fig. 4, polar views of equatorial plates; second meiotic division. Fig. 5, macrospore mother cell at diakinesis. Fig. 6, pollen mother cell, lateral view of equatorial plate; first meiotic division. Fig. 7, macrospore mother cell, lateral view of equatorial plate; first meiotic division. Fig. 8, anaphase. All approximately × 2800.

pair of chromosomes in *Zanthoxylum americanum*, as was reported by SINOTÔ (2) and NAKAJIMA (1) for *Z. piperitum*.

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BRIEFER ARTICLE

MACERATION METHOD TO DEMONSTRATE THE VASCULAR SYSTEM IN ZEA MAYS

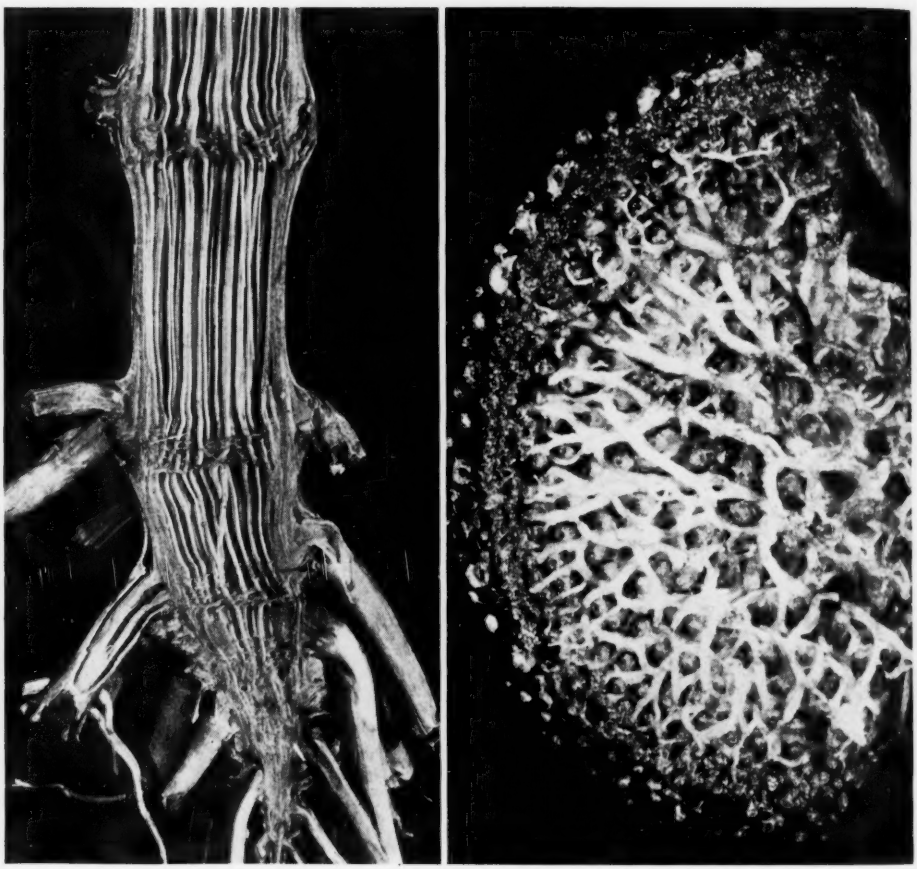
(WITH TWO FIGURES)

The maize axis has long been used as a monocotyledonous stem type, and generations of students have examined it in transverse section. When the question of the course of the bundles is raised, however, few can give even a general description, especially of the behavior at the node, since it is well nigh impossible to elucidate the construction by serial sections. A note by EVANS[1] caused the writer to work out the following method whereby abundant class material of completely separated vascular skeletons may be simply and permanently prepared (fig. 1). The same method could probably be applied to other material; the writer has even used it successfully on sugar-cane stem which had been preserved in formalin-acetic-alcohol.

The method relies on the fact that it is possible to remove the parenchyma and ground tissues by rotting and yet leave intact the vascular bundles and highly lignified parts. Mature maize plants, after careful removal from the soil, have the leaves and roots trimmed to within about $\frac{1}{4}$ inch of the axis, care being taken to leave the epicotyl and the first few internodes. The stem is now washed completely free of soil and then split into two, preferably in the plane of the leaves. The splitting is best done by starting at the epicotyl with a scalpel, and when the wider parts of the axis are reached, changing to a stronger knife or even to a chisel and mallet if the specimen is large and well matured. Some of the upper part of the stem is now removed, since for class use a whole axis is unwieldy and there is no need to include more than one or two nodes above the region from which arise the highest roots. The specimen should be trimmed to its final size and shape at this stage, as it is much more difficult to do this on the finished skeleton. If, when the axis is shortened, the cut is made above the node, the nodal anastomosis will be clearly displayed in the final preparation (fig. 2).

The prepared halves are now placed in any convenient receptacle which is not too deep, covered with tap water, and allowed to decompose. Rotting of the ground tissue is hastened if the water is kept warm (about 70° F.). The addition of ten to twenty crushed peas or maize grains also seems to hasten the process, probably by insuring rapid initial increase in the number of bacteria. Additional water is necessary from time to time to replace that lost by evaporation. The material must always be completely covered, since if any part remains above the liquid, dark stains—which are impossible to remove—result from the growth of molds just above the liquid surface. After a few weeks, the time depending largely on the temperature of the water, the parenchyma

[1] EVANS, A. T., Vascularization of the node in *Zea mays*. BOT. GAZ. **85**:97–103. 1928.



FIGS. 1, 2.—Fig. 1 (left), base of maize stem split longitudinally and then "retted." Fig. 2 (right), network of anastomosing bundles at node.

will have rotted and the center of the stem feel soft and gelatinous. A strong jet of water will now free the vascular bundles from the ground parenchyma. The washing is conveniently done by using a jet from a piece of rubber tubing, which allows wide control of the volume and rate of flow of the water. After the center has been freed from debris and a strong jet forced through the nodal regions in all directions, a jet is put on the exterior, cleaning up the outside and usually removing the internodal epidermis in large sheets. The outer bundles do not fall apart, as they are all imbedded in the subperipheral sclerenchyma band. If the rotting is not complete, the stem is cleaned up as much as possible and then returned to the tank for another week or so.

The skeleton is best kept as a dry specimen. It should be placed in 90 per cent alcohol for a day or two and then allowed to dry at room temperature, a process which takes about 12 hours. The alcohol serves to remove the unpleasant smell which the specimen has when first taken out of the tank and also to enable the specimen to be dried without much shrinkage or collapse.

When finished, the skeleton, which should be free from odor and almost white in color, is strong, durable, and well able to stand up to the handling received in class use.—B. C. SHARMAN, *Botany Department, The University, Leeds 2, England.*

CURRENT LITERATURE

Compuestas Bonaerenses: Revisión de las Compuestas de la Provincia de Buenos Aires, la Capital Federal y la Isla Martín García. By ANGEL L. CABRERA. Extracted from the Revista del Museo de La Plata (Nueva Serie) Tomo IV, Sección Botánica. 1941. Pp. 450. Figs. 145. Pls. 10.

A study is presented "of all the species of Compositae found in the Province of Buenos Aires, in the Federal District (City of Buenos Aires) and in the Island of Martín García which lies in the Río de La Plata." In the entire country of Argentina the Compositae, with about 200 genera and about 1200 species, constitute approximately one-sixth of the phanerogamic flora. In the restricted area studied, 102 genera, containing in all 299 species, are found to occur. Of these, "74 genera and 245 species are native. The rest are adventitious or adapted from cultivation."

The author presents full and carefully drawn descriptions, together with lists of specimens studied and often with critical observations. The numerous figures are mostly full-page, carefully executed etchings, many of them by the author himself. These should prove of great value, especially in the case of the less well-known species. The ten plates are given over to twenty habit-photographs of selected species. Keys are given to the various tribes as well as to the component genera and species. The Astereae, Inuleae, and Heliantheae are seen to be the best represented tribes, while the genera with the most species are *Eupatorium* (19), *Baccharis* (29), and *Senecio* (28). Several new species and varieties are described and there are a few new combinations. A bibliography of 118 citations (pp. 433–437) and a concise summary in English (pp. 438–439) are appended. The book as a whole represents a noteworthy contribution to the literature on Compositae.—E. E. SHERFF.

The Structure of Protoplasm. Edited by WILLIAM SEIFRIZ. Ames, Iowa: Iowa State College Press, 1942. Pp. vii+283.

One of the most important summaries of protoplasmic structure in the English language, this monograph is the first to be published by the American Society of Plant Physiologists. It contains the papers presented before the organization at Philadelphia in December, 1940. The original symposium was arranged by Dr. SEIFRIZ, and the volume is edited by him. Much of the excellence of arrangement and the integration of the symposium depended upon his keen analysis of the field and his choice of men to handle each subject. Following the introduction, written by SEIFRIZ, these topics are discussed: Microscopic structure of the cell wall, by C. W. HOCK; Proteins and protoplasmic structure, by L. W. MOYER; Molecular structure in protoplasm, by O. L. SPONSLER and JEAN D. BATH; Some mechanical properties of sols and gels and their relation to protoplasmic structure, by HERBERT FREUNDLICH; Structural differentiation of cytoplasm, by G. W. SCARTH; Structural differentiation of the nucleus, by C. L. HUSKINS; Protoplasmic streaming in relation to gel structure in the cytoplasm, by D. A. MARSLAND; Relation of the viscosity changes of protoplasm to amoeboid locomotion and cell division, by W. H. LEWIS; Physical aspects of protoplasmic streaming, by NOBURÔ KAMIYA; and Some physical properties of protoplasm and their bearing on structure, by WILLIAM SEIFRIZ. A short supplement contains a brief communication on protein and protoplasmic structure by K. H. MEYER, of Geneva, and a letter from W. T. ASTBURY, of Leeds, England.

The paper by FREUNDLICH was the last thing he wrote before his death. It will be prized by his admirers as will also be the fine portrait which appears as a memorial to him.

The material is well illustrated, and the individual papers carry literature lists which add much to the usefulness of the volume. The press work has been beautifully done under the watchful eyes of Dr. W. E. LOOMIS, of Iowa State College, who provides the foreword.

Its up-to-date points of view, and the documentary evidences of structure presented, make this monograph an indispensable work for all students and investigators of protoplasmic phenomena.—C. A. SHULL.

Advances in Colloid Science. Edited by E. O. KRAMER and collaborators. New York: Interscience Publishers, Inc., 1942. Pp. xii+434. $5.50.

The editors have planned a series of volumes on the advances of colloid science, to appear whenever conditions warrant publication. This first volume of the series contains a dozen monographic reviews with the following titles and authors: The measurement of the surface areas of finely divided porous solids by low temperature adsorption isotherms, by P. H. EMMETT; The permeability method for determining specific surface of fibers and powders, by R. R. SULLIVAN and K. L. HERTEL; A new method of adsorption analysis and some of its applications, by ARNE TISELIUS; Solubilization and other factors in detergent action, by J. W. MCBAIN; Recent developments in starch chemistry, by K. H. MEYER; Frictional and thermo-dynamic properties of large molecules, by R. E. POWELL and HENRY EYRING; The constitution of inorganic gels, by H. B. WEISER and W. O. MILLIGAN; The creaming of rubber latex, by G. E. VAN GILS and G. M. KRAAY; Streaming birefringence and its relation to particle size and shape, by J. T. EDSALL; Synthetic-resin ion exchangers, by R. T. MYERS; The study of colloids with the electron-microscope, by T. P. ANDERSON; and Anomalies in surface tensions of solutions, by R. A. HAUSER.

Each monograph carries its own bibliography, in some cases short lists but in others over 100 references. They are written in clear style and will prove exceedingly helpful to anyone interested in these rapidly progressing fields of investigation and application of science to practical problems. The editors deserve congratulations for the initiation of this series of reviews, a number of which come from European laboratories. Many of the reviews contain material of special significance to physiological investigations.—C. A. SHULL.

Desert Wild Flowers. By EDMUND C. JAEGER. Stanford University Press, 1941. Pp. 30+322. Illustrated. $3.50.

First published in 1940, this book has now been revised by the inclusion of an artificial but workable key to the 764 species of California desert plants which are illustrated by line drawings (a few by photographs). It should now have an even wider appeal to all nature lovers in this area, who may identify easily and rather positively most of the plants which they are likely to encounter. No grasses are included.—C. E. OLMSTED.

Soils and Fertilizers. By FIRMAN E. BEAR. New York: John Wiley and Sons, Inc., 1942. Pp. xiii+374.

The third edition of this book, formerly entitled *Soil Management*, which has just appeared, has been revised and improved in various ways. Although some of the headings have been changed slightly, the twenty-six chapters follow the same order and treat the same topics as in earlier editions. It is commended especially to beginners in the study of soils and fertilizers, for it is simply and concisely written and deals with the principles without confusing them with too much detail. It is a very good elementary text in its field.—C. A. SHULL.